The University of Wisconsin Library

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in The University of Wisconsin Library are open for inspection, but are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages may be copied only with the permission of the authors, and proper credit must be given in subsequent written or published work. Extensive copying or publication of the thesis in whole or in part requires also the consent of the Dean of the Graduate School of The University of Wisconsin.

This thesis by CHARLES LEWIS STEBBINS

has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

A Library which borrows this thesis for use by its patrons is expected to secure the signature of each user.

NAME AND ADDRESS DATE

THE EFFECT OF EXERCISE ON MORPHOLOGICAL CHANGES ASSOCIATED WITH SENILE MUSCULAR ATROPHY

A thesis submitted to the Graduate School of the University of Wisconsin-Madison in partial fulfillment of the requirements for the degree of Doctor of Philosophy

by

Charles Lewis Stebbins

Degree to be awarded: December 19 81 May 19____ August 19____

Approved by Thesis Reading Committee:

William Reddan — Major Professor

8/20/81 — Date of Examination

Irwin J. Engle

Edward Schultz

Robert M. Bock — Dean, Graduate School

THE EFFECT OF EXERCISE ON MORPHOLOGICAL CHANGES ASSOCIATED WITH SENILE MUSCULAR ATROPHY

by

CHARLES LEWIS STEBBINS

A thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

(Physical Education)

at the

UNIVERSITY OF WISCONSIN-MADISON

1981

AWB
S81102
C436

ACKNOWLEDGEMENTS

I would like to take this opportunity to express sincere appreciation to Dr. W. Reddan for serving as my thesis committee chairman; to Dr. R. G. Cassens for his technical assistance and for graciously allowing me the use of his facilities; to Dr. E. Schultz for his patience and constructive criticism; and to Dr. F. S. Nagle, and Dr. H. Montoye for their kind assistance while serving on my thesis committee.

Much valuable help and guidance in the inception and completion of this investigation was willingly offered by Dr. E. L. Smith for which I am very grateful.

A special word of thanks goes to Ron Smith for his tireless efforts in the organization and implementation of the training program and to Tim Musch for his help with the muscle extraction.

This investigation was supported by the Graduate School, University of Wisconsin-Madison, and by a pilot study-predoctoral grant from the National Institute on Aging, #127R.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	iv
LIST OF FIGURES	v
LIST OF PLATES	vi
ABSTRACT	vii
CHAPTER I: INTRODUCTION	1
CHAPTER II: REVIEW OF LITERATURE	8
CHAPTER III: MATERIALS AND METHODS	32
CHAPTER IV: RESULTS	46
CHAPTER V: DISCUSSION	60
CHAPTER VI: SUMMARY AND CONCLUSIONS	82
BIBLIOGRAPHY	87
APPENDIX	97

LIST OF TABLES

Table		Page
I	Training Schedule of the Experimental Group..	37
II	Mean Body Weight, Heart Weight, and Heart Weight/Body Weight Ratio.....	54
III	Mean Gastrocnemius Weight, Mean Soleus Weight, and Ratio of Gastrocnemius and Soleus Weight to Body Weight.....	55
IV	Type I and Type II Fiber Diameters and Type I/Type II Fiber Diameter Ratios.....	56
V	Mean Percentage of Small Diameter Fibers.....	57
VI	Mean Fiber Type Percentages.....	58
VII	Mean Percentage of Growth Configurations and Mean and Pooled FTIR's and ATIR's.....	59

LIST OF FIGURES

Figure		Page
I	Animal Housing Facilities.....	33
II	Motor Driven Exercise Treadmill.....	35

LIST OF PLATES

Plate		Page
I	Photomicrographs of Isolated Small Diameter Fibers.....	52
II	Photomicrographs Depicting Various Examples of End Plate Sophistication and Nerve Terminal Branching.....	53

THE EFFECT OF EXERCISE ON MORPHOLOGICAL CHANGES ASSOCIATED WITH SENILE MUSCULAR ATROPHY

BY

Charles Lewis Stebbins

Under the Supervision of Associate Professor
William G. Reddan

ABSTRACT

The present study investigated the effects of chronic treadmill running on the morphology of skeletal muscle in aged female rats. Small diameter percentage, fiber type percentage, and motor end plate growth configurations (terminal sprouting) were examined. Twenty, 21-month-old rats were exercised for 20 weeks on an exercise treadmill. The animals were exercised five times a week, and the intensity and duration of the running was progressively increased to 23 meters/min (8° incline) for 30 minutes.

Following the training program, at 26 months of age, the animals were sacrificed and their soleus and gastrocnemius muscles were extracted. To examine aging and exercise effects, the same muscles were excised from fifteen 21-month (baseline) and fifteen 26-month (control) old animals for comparison.

Endplate and muscle fiber morphology were demonstrated by staining for both myosin ATPase and acetylcholinesterase activity. The soleus showed greater exercise induced changes than the gastrocnemius. The exercise soleus group displayed a greater percentage of Type I fibers than either the baseline or control groups. This was interpreted as a conversion of fiber type. The incidence of nerve terminal sprouting was greater in the soleus exercise group than in the control group. The terminal sprouting in the control

group was in turn greater than the baseline group. The only significant difference among the gastrocnemius groups was with respect to the percentage of small diameter fibers. The exercise group showed a greater percentage than the baseline group but less than the control group.

The following conclusions were reached: (1) age induced transformations in endplate morphology precede degenerative changes in muscle fibers; (2) postural muscles, such as the soleus, demonstrate greater adaptive changes (conversion of fiber types and terminal nerve sprouting) to aging and training than phasic muscles such as the gastrocnemius; and (3) the aerobic activity utilized in the present study did not have a negative effect on the skeletal muscle of the experimental animals (such as enhanced muscle degeneration) as reported in previous studies.

Approved by:

William Reddan

CHAPTER I

INTRODUCTION

The process of aging is a progressive development that might be conceptualized as the conclusion of ontogenesis. It is a process which affects all tissues and organs of the body, although the onset and rate of progression varies from one tissue to another. The phenomenon of senile muscular atrophy is of particular interest for study because the age induced changes in the neuromuscular system have a profound effect on muscular strength and endurance as well as on the general functional capacity of the organism (42, 43, 44, 66, 85).

Senile muscular atrophy involves degenerative biochemical, morphological, and physiological changes which take place within skeletal muscle and the peripheral motor nerves (6, 15, 33, 34, 40, 41, 43, 44, 75, 83, 94, 95). These changes are not distinct nor separate from one another and the key to understanding the entire process may lie in the knowledge of how these changes are interrelated.

Attempting to characterize the process of senile muscular atrophy is difficult at best. The decline in motor function begins early in life, probably before any morphological evidence can be observed (61, 81). Additionally, maximal functional capacity of the

neuromuscular system peaks early in the life span of most species (68). This decline in motor performance is very complex. Gutmann and Hanzlikova (44) suggest that the source of this decline encompasses changes in motivation, receptors, effectors, central synaptic mechanisms, and neural pathways. However, they conclude: "It is evident that an attempt to understand basic mechanisms must center on cellular age changes; however, the nature of these changes is not yet clarified by clear evidence regarding origin, extent or effects of such changes (p.327)".

An additional problem in characterizing the aging process in skeletal muscle stems from the fact that muscle fibers do not represent a homogeneous tissue and the state of the fibers at any given time depends on the influence and presence of many trophic influences (66). As a result, separating specific age dependent muscle changes from ancillary factors induced by other body systems, such as the nervous, cardiovascular, and endocrine systems, presents a challenging technical problem (33, 66).

A review of the published studies on senile muscular atrophy tends to illustrate this problem. With respect to enzyme changes Rothstein (81) summarizes the situation clearly: "...in a few cases, the activity of the same enzyme is reported to go up, go down or stay the same depending upon the investigator, the animal, or the tissue being studied." Thus, it appears that any study of the

process of senile muscular atrophy must incorporate a methodology which is clear and concise, consistent in design, and accountable for the influence of secondary systems and factors.

To date, relatively few studies have been undertaken to determine the temporal sequence of degenerative morphological changes in skeletal muscle (rat or human). Because of their relatively short life span and their manageable size, the rat has been used extensively as a model for studying the changes in skeletal muscle function associated with aging (66). Studies employing the rat as the experimental animal have generally used a design whereby young adult rats (12-14 mos.) are compared morphologically to older rats (24 mos. +). The results have been complicated by the fact that grouping of young and old rats has not been standardized. On occasion, 12-18 mo. adult rats have been utilized in aged groups and young animals (1-3 mos.) have been used as adult controls (33). Additionally, it appears that no studies have sampled at monthly intervals from 16-24 months in an attempt to establish what specific changes in morphology take place at what specific ages. Tauchi et al. (92) using micromeasuring techniques, found a decrease in weight, number and volume of skeletal muscle when comparing rats 12 months of age to those 24 months of age. McCafferty et al. (65) examined muscle and organ weights of rats 3, 5, 10, 15,

25, 30, and 36 months of age. They observed no decrease in either organ or muscle weight until the twenty-fifth month. Decreases in size, number and weight of skeletal muscle have been documented as some of the more readily detectable characteristics of senile muscular atrophy. This has been observed in both human and rat skeletal muscle (44, 65, 92, 99). Based on the studies by McCafferty et al. and Tauchi et al., it is probable that the threshold for manifestation of senile muscular atrophy in the rat is at some point after 20 months of age, but before 24-25 months.

When examining the research that has been directed towards the phenomenon of senile muscular atrophy, it becomes apparent that the majority of these investigations have been concerned with biochemical, physiological, and morphological descriptions of the process in an attempt to pinpoint the source of this degeneration. Results from these studies have given rise to the theory that senile muscular atrophy appears to be initiated by a gradual loss of neuromuscular contact by individual muscle fibers (38, 39, 40, 43, 44, 45, 46). This occurs primarily at the neuromuscular junction and may in turn lead to functional and morphological degeneration within and among the muscle fibers. These changes include: (1) an increase in small diameter fibers, believed to represent degenerating fibers (97); (2) an increase in type I/type II fiber diameter ratios (98); (3) an increase in the percentage of type I

fibers, believed to represent a selective atrophy of type II fibers (61, 62, 63, 98); and (4) an increase in growth configurations at the motor endplate, which may be an attempt to compensate for loss of neural contact.

While pinpointing the source or sources of senile muscular atrophy is an important goal, it also seems important to investigate potential measures that might delay the onset of the process, slow its progress, or even stimulate collateral regeneration (as seen following nerve crush or denervation) (20). One such preventive measure may be exercise. It has been shown that individuals who maintain a high level of aerobic activity into old age tend to demonstrate a higher functional work capacity and live longer than their more sedentary counterparts (55). Slower aging seen in tissues of wild animals when compared to their domesticated counterparts and the observation of increased life spans in caged rats with access to free wheel exercise (in relation to sedentary controls), also point to a possible interrelation between exercise and the aging process (37, 47). Additionally, exercise is instrumental in the reversal of disuse atrophy seen in bed ridden patients, a phenomenon very similar morphologically to senile muscular atrophy (26, 97). Based on these observations, the present investigation was initiated to examine the effects of exercise on the course of selected degenerative changes

associated with senile muscular atrophy. A particular concern of this study was the effects of exercise introduced late in the lifespan of animals that have led otherwise sedentary lives. These effects and responses remain largely uninvestigated.

STATEMENT OF PROBLEM

Little evidence exists as to the possibility of slowing the onset of or delaying the progress of the degenerative changes associated with senile muscular atrophy. The purpose of this study was to evaluate the effect of exercise (interpolated late in the life span but prior to the visible manifestations of senile muscular atrophy) on selected morphological changes associated with senile muscular atrophy. More specifically, this investigation compared a group of trained experimental rats with sedentary control animals of the same age for differences in: (1) type I/type II fiber diameter ratios and fiber percentages; (2) small diameter (necrotic) fiber percentages; and (3) collateral regeneration, evident by evaluation of motor end plate growth configurations, functional terminal innervation ratios (FTIR) and absolute terminal innervation ratios (ATIR). The following hypotheses have been developed for the purpose of studying this problem:

1. There is no difference in type I/type II fiber

diameter ratios or type I/type II fiber percentages in aged skeletal muscles following exercise training.

(H_0: $a_1 = a_2$)

2. There is no difference in the percentage of small diameter (necrotic) fibers in aged skeletal muscle following exercise training.

(H_0: $b_1 = b_2$)

3. There is no increase in collateral regeneration or growth configurations in aged skeletal muscle following exercise training.

(H_0: $c_1 = c_2$)

In the event of rejection of any or all of the proposed hypotheses, the foregoing alternative hypotheses have been adopted:

1. H_1: $a_1 \neq a_2$
2. H_1: $b_1 \neq b_2$
3. H_1: $c_1 \neq c_2$

CHAPTER II

REVIEW OF LITERATURE

Senile changes in morphology of cross striated skeletal muscle

Tauchi et al. (92), McCafferty et al. (65), and Rowe (80) have reported decreases in weight, volume and size of muscle fibers in aging rats and mice. It has also been reported that the decrease in weight found, in white fibers (Type IIa and IIb) is due primarily to a decrease in muscle volume, while in red fibers (Type I) the decreases in weight are due largely to a decrease in the number of fibers (92). Larsson et al. (61, 62, 63), and Tomonaga (98) and Scelsi et al. (83) have reported a general grouped atrophy of type II fibers with a lesser degree of atrophy in type I fibers among aging men. These phenomena have also been confirmed physiologically in electromyographic studies (16, 18, 102). Tomonaga additionally found an increase in the ratio of typeI/typeII fiber areas. Increases in the percentage of type I fibers with age have also been reported (73, 94, 95). Jennekens et al. (54) found an increased degree of variation in fiber size in aged striated muscle. Sometimes this was due to a random atrophy of both type I and type II fibers and sometimes to an atrophy of type II fibers alone.

Similar results have been observed in rats and mice (6,

16). Caccia et al. (16) reported striking changes in the percentages of muscle fiber types among young (3 mos.), middle ages (19 mos.) and old (30 mos.) rats. Examination of the soleus (SOL) and extensor digitorum longus (EDL) muslces in all three groups revealed three distinct fiber types. The SOL muscles from the young and middle aged rats demonstrated the presence of only slow oxidative (SO) (Type I) and fast oxidative glycolytic (FOG) (Type IIa) fibers. The EDL muscles from the young, middle aged and old groups demonstrated the presence of FOG, SO and fast glycolytic (FG) (Type IIb) fibers. The SOL muscles demonstrated the presence of a fourth fiber with myofibullar ATPase activity intermediate between SO and FOG fibers were designated intermediate fibers.

Comparisons of fiber type percentages in the EDL showed a gradual increase in SO (21% young to 13.24% old) and FOG fibers (24.9% to 44.6%) and a decrease in FG fibers (73.0% to 42.2%) with age. The trend in the SOL was quite different. The SO fiber percentage increased in the middle age group (from 73.5% to 93.5%) and demonstrated a decrease in the old group (40.5%). The trend was just the opposite for the FOG fibers. The middle age group showed a marked decrease in percentage when compared to the young group (22.2% to 4.2%) but the old group showed the highest percentage of the three (43.7%). The intermediate fibers were a measurable factor in the old group only (15.8%).

Based on histological data and evidence from stimulation recording of contraction time and relaxation time, the authors suggested a redistribution or conversion of muscle fibers during aging that accompanies an overall loss of the total number of fibers. The pattern may involve an initial loss of "fast twitch" fibers followed by or coinciding with a conversion from "fast twitch" to "slow twitch."

From a functional point of view, Larsson et al. (63) found that increases in type I/type II fiber ratios seen in aging muscle (due primarily to type II fiber atrophy) correlated significantly with decreases in dynamic and isometric strength. Type II fibers are apparently more susceptible to atrophic changes in size than type I fibers (54, 83). Larsson et al. also hypothesized a decrease in both isometric and dynamic endurance. These decreases were concurrent with the increase in type I/type II fiber ratios, when measured in absolute terms. Aniansson et al. (3) found comparable results after assessing maximal static and dynamic strength in elderly men and women. Additionally, they observed a deterioration of motor coordination, an observation they felt may or may not have been functionally related to a reduction in type II fiber area.

Another aspect of senile muscular atrophy is an increasing occurrence of small diameter muscle fibers. Tomlinson et al. (97) have observed and suggested that

reductions in the diameter of muscle fibers and the marked variations in their size are major characteristics of both disuse and senile muscular atrophy. It has also been observed that the small fibers are collected in groups of 3 to 10, often around other fibers which are "more normal." Furthermore, the shrinkage of these fibers is not correlated to any motor unit pattern or subunit pattern. Thus it appears to be separate from the aforementioned grouped atrophy. Tomonaga (98), among others, has suggested that these small fibers are an important step in the process of fiber degeneration, especially since the fibers appear angulated and dense. This may represent some form of necrosis.

In what appears to be a related development, a nonuniform pattern of muscle degeneration has been observed both between muscle types and between groups of fibers within a given muscle (44, 83). There is a loss of the mosaic pattern of muscle when a cross section, stained for myosin ATPase or NADH diaphorase activity, is examined under a microscope. Gutmann et al. (44) contend that this "de-differentiation" is a result of a loss of heterogeneity of the different muscle types, probably due to a decrease in all enzyme activities associated with energy metabolism. This is particularly evident in the relation of glycolytic to oxidative enzyme activities in the affected muscle.

The pathogenesis of the "mosaic" pattern of

degeneration is unknown. Fujisawa (35) strongly suggests that neurogenic factors play a major role in the production of not only the mosaic degeneration, but also the simple grouped atrophy discussed earlier. Jennekens et al. (54) found evidence of neurogenic factors in aged skeletal muscle demonstrating atrophy of both type I and type II fiber types. This included uniform grouping of atrophied fibers and the presence of target fibers. Gutmann et al. (43, 44) offer additional evidence implicating nervous factors in the "de-differentiation" process. They believe that the role of these factors is illustrated by the loss and recovery of differentiation of muscle fiber types after denervation or reinnervation of muscle. Roberts et al. (78) comment that the process of mosaic degeneration may result from age induced changes in muscular innervation.

The de-differentiation process or mosaic pattern of degeneration is observed under the light microscope due to changes in staining response of myosin ATPase or NADH diaphorase activity. Changes in the activity of not only myosin ATPase but of all enzyme activities concerned with energy metabolism are seen in aging skeletal muscle (6,44). Changes vary depending on the fiber type involved.

Bass et al. (6) demonstrated these differential changes in an examination of aged rat extensor digitorum longus (EDL) (predominantly fast twitch fibers) and soleus

(predominantly slow twitch fibers) muscles. The EDL showed a decrease in the activity ratio of glycolytic enzymes to oxidative enzymes, when compared to values obtained for young rats. However, there was a concomitant increase in the enzymes concerned with glucose phosphorylation. As a result, the EDL appeared to have increased its reliance on aerobic oxidation and its role of glucose oxidation. The soleus muscle, in contrast to the EDL, demonstrated a relatively unchanged activity ratio of glycolytic to oxidative enzymes. There was, however, some decrease in the activity of the glycolytic enzymes, but this appeared to be offset by a concurrent decrease in oxidative enzymes.

Bass et al. concluded that there is a differential trend in the decrease of enzyme activities in type I and type II fiber types. They suggested that this finding was consistent with histochemical type grouping and preferential type II fiber atrophy which also characterizes senile muscular atrophy. They also concluded that a general decrease in muscle strength results from the decreases in total aerobic and anaerobic enzyme activity. They additionally postulate a dominance of neurogenic factors in this overall atrophic process.

In conclusion, comparisons of studies using rats as experimental subjects, with those using human subjects have shown many consistent parallels of morphological,

biochemical, and functional changes in aging skeletal muscle (6, 15, 32, 61, 65, 83, 94, 95, 97, 98). This may indicate similar mechanisms of aging in both species.

Senile Changes Of Nervous Origin

The role of nervous control in relation to the structure and function of skeletal muscle is complex. It is concerned with synaptic transmission, excitation and contraction coupling, which lead to muscle contraction. Furthermore, the differing membrane, structural, contractile and metabolic properties among muscle fibers are tightly related to the physiological properties of the motor nerve cells and the nerve fibers which innervate them (43, 44). Thus, it is important to be aware of the fact that degenerative changes of nervous origin will probably effect the integrity of the associated muscle fibers.

Gutmann et al. (39) attempted to determine if the atrophy and metabolic disturbances observed in muscle during aging could be related to loss of alpha motor neurons (a central effect) or to atrophy and/or loss of individual muscle fibers (a peripheral effect). Comparing 4 and 24 month old rats, he observed no loss of alpha motor neurons in the spinal cord of the older rats. He did, however, observe a 33% decrease in the size of the motor unit. This decrease in the number of motor nerve branchings paralleled

a decrease in total muscle fibers. Gutmann et al. concluded that the decrease in motor unit size was probably due to a random degeneration of the end plates, resulting in a loss of muscle fibers. Gutmann et al. supported this contention with the observation of decreased acetyl cholinesterase activity in the senile muscles. Gutmann had also reported this finding in a previous study (38).

Using human subjects, McComas et al. (67) and Brown (14) found evidence of a decrease in the number of alpha motor neurons as well as a decrease in the size of the motor unit. The studies used electro-physiological methods for determining the number of functional alpha motor neurons rather than direct counting methods. Both studies concluded that there was little decrease in the number of functional motor units through the first six decades of life. Beyond 60 years of age, however, even healthy individuals experienced a progressive and marked loss of motor neurons. Many individuals had fewer than one half the number of units present earlier in life. Brown postulated that the results obtained by Gutmann et al. (aging decreases in just the size of the motor unit) may have "represented an earlier stage of 'primary neuronal cell failure,' characterized by uneven and scattered degenerative changes in terminal branches of the motor axon, with a consequent reduction in the population of innervated muscle fibers in each motor unit" (14, p. 207).

In relation to the cause of type II grouped atrophy,

characterized by an increase in type I/type II fiber area ratio, Tomonaga (98) suggests a process of functional denervation. He contends this is due to a decrease in trophic factors of the neuron which are probably a result of irregularities in innervation of individual muscle. This apparently leads to loss of neuromuscular contact.

It has been reported by Tomlinson et al. (97) that the phase of senile muscular atrophy characterized by an increasing occurrence of random or small grouped fibers may be an additional end result of denervation. This denervation is also associated with a loss or degeneration of the peripheral fine fiber branches of the alpha motor neuron. Tomlinson refers to this phenomenon as "neurogenic" muscular atrophy.

Gutmann et al. (38) found random degeneration and regeneration of some end plates in the aged rat. Compensatory outgrowths were observed in axons losing neuromuscular connection. These outgrowths characteristically terminated in thick rather than the fine branchings seen in normal end plates. Staining for cholinesterase activity outlined the borders of the end plate. Using this technique Gutmann et al. reported diminished control of acetylcholine in the older animals. This finding, along with the observation of random degeneration of end plates, prompted Gutmann et al. to suggest a disturbance of neurotrophic relation

between nerve and muscle as an important element of aging in skeletal muscle.

Gutmann et al. (45, 46) have described the apparent stages of this neurogenic atrophy or functional denervation. Initially the neuromuscular junction is unimpaired, but there is a marked decrease of neurotransmitter release. This takes place in spite of the absence of axonal degeneration. This phenomenon is often referred to as one of "silent synapses," since neuromuscular contact is still intact. A decrease in neurotrophic factors apparently precipitates the decreased integrity of the neuromuscular junction. The final stage in this functional denervation process is characterized by disruption of the muscle membrane, and its infoldings, and a pulling away of the terminal axon from the muscle fiber. Since the synaptic vesicles can still be observed, this phenomenon is characterized as one of "silent axon terminals."

Other neurogenic aspects of senile muscular atrophy have been reported by various authors. Gutmann et al. (38, 42) examined aged end plates and characterized those believed to be degenerating as demonstrating granular fragmentation, long rows of nuclei and increased numbers of muscle nuclei in the end plate region. Ultrastructural changes believed to characterize functional denervation were also reported by Gutmann et al. (42). These changes included an expansion of the primary synaptic cleft,

aggregation of synaptic vesicles, neurotubules and filaments, an enlargement of the basement membrane, and unusual branching of the junctional infoldings. These changes occurred in the absence of any observable axonal degeneration or direct denervation at the end plates. Coers et al. (20) have reported some general degenerative end plate changes seen in various pathologies and in denervation. These included swelling of the terminal expansions of the end plate, which occasionally fuse into a single mass, and a degenerative swelling of the subneural apparatus. Van Steenis et al. (101) reported the presence of a primary demyelinating process in the peripheral nerves of 34 month old rats. This process was characterized by apparently normal axons with degenerating myelin sheaths demonstrating swelling, dilatation and the appearances of macrophages within the dilated sheaths. Degenerative changes observed in the musculature of these aged animals were believed to be secondary to neurogenic changes observed in the peripheral nerves. Gutmann et al. (40, 41, 42) suggest that the most important element involved in maintaining the structure and functional capacity of the muscle is the trophic functions of the nerve cell. They also contend that the decline in trophic function with aging is primarily a result of deficiencies in proximo-distal axon flow of proteins. This leads to a random degeneration of axons and a decreased

control over the muscle.

The peripheral nervous system is able to compensate functionally for these degenerative changes, whether they are precipitated by disease, aging, and/or degeneration (functional or direct). This compensatory process, referred to as collateral nerve regeneration, has been discussed at length by many investigators (4, 14, 20, 21, 22, 58, 59). The fact that it occurs is generally accepted, but there is some discrepancy as to the relation of the exact mechanisms to the compensatory effects that are observed in senile muscular atrophy.

Edds (30) has described the collateral regeneration that follows the partial denervation of muscle. The process usually begins within four days of the partial denervation. It is initiated by the occurrence of delicate collateral sprouts on the motor axons. They are usually observed within 200 microns of the end of the parent fiber. Collateral sprouts are non-myelinated outgrowths from a nerve fiber which usually terminate in a growth cone at or near the muscle fiber innervated by the parent terminal. The sprouts slowly migrate towards the denervated end plates. Once connected with the end plate they begin to mature and within about two months they achieve a normal size and diameter. Another common characteristic of this reinnervation process is the development of patterns of outgrowth or collateral branches which eventually rein-

nervate adjacent muscle fibers.

Barker et al. (4) have described a similar process to explain the replacement of aging end plates. The early stages of this replacement process are similar to the process described by Edds. The unmyelinated sprout is an outgrowth from an aging end plate. However, instead of making contact with the degenerating end plate, the sprouts make contact with the muscle adjacent to the parent ending and initiates the formation of a new end plate. Once the sprout matures and myelinates, the parent end plate completely breaks up and removal of the aged ending and its terminal branch takes place.

A different process of compensation for the functional denervation seen in senile muscular atrophy was reported by Tuffery (99). When comparing young (6 month) and old (6, 10, 18 years) cats, he found that the older animals demonstrated a higher incidence of growth configurations than the younger animals. These growth configurations were characterized by collateral sprouting and contributions. Contributions are similar to sprouts. They are observed as nonmyelinated outgrowths from a node. However, they are seen terminating in an axon terminal on the same end plate as the parent ending rather than terminating in a growth cone prior to making contact with a muscle fiber. As a result of these outgrowths, many complex end plates were

observed in the older animals. These complex end plates were characterized by two or more myelinated branches of the terminal axon ending on the same sole plate.

Tuffery found all growth configurations to be in contact with the sole plate of the parent ending. He observed no evidence of the end plate replacement process described by Barker, nor did he observe any parent end plates breaking up and being removed. Tuffery suggested the theory of "end plate elaboration" to account for increasing end plate complexity during aging. According to this theory, the increased growth configurations are a response of the neuron to a decline in trophic function, leading to progressive degeneration and denervation. The growth configurations are an attempt to increase the use of the area of neuromuscular contact and maintain function.

This contention was supported by results of Pestronk et al. (75) who elicited nerve terminal branching (growth configurations) by "pharmocological denervation." This was brought about by botulinum toxin injections. The botulinum toxin treatment stimulated strikingly marked increases in end-plate length and terminal aborization in 2, 10, and 18 month old rats.

The phenomenon of collateral branching may be another important functional compensation seen in senile muscular atrophy. Brown (14) suggests that functional compensation for motor unit loss in old animals is probably accomplished

through the reinnervation of denervated muscle fibers by sprouting from neighboring healthy axons. The extent of this compensation is quite remarkable. According to Brown, over 80% of the motor units may disappear before there is obvious wasting or weakness.

Coers et al. (20) contend that it is relatively uncommon to observe collateral branching in the nerve bundles of normal adult muscle. As a result of this observation, diagnosis of denervation can be safely diagnosed where collateral branching is plentiful. If the denervation or loss of motor units is severe and the stimulus to surviving axons is vigorous, a single nerve fiber may initiate many collateral sprouts, each one reinnervating a previously denervated muscle fiber.

As evidence of collateral branching in muscle samples, functional (FTIR) and absolute (ATIR) terminal innervation ratios are calculated (9, 20, 21, 22). The FTIR is the ratio of the number of muscle fibers innervated by a given number of subterminal axons to the number of subterminal axons. The ATIR is the ratio of the number of motor end plates innervated by a given number of subterminal axons to the number of subterminal axons. The average human value for FTIR is 1.09/1 while the average value for ATIR is 1.13/1. Increases in FTIR and ATIR above normal are accepted as clear evidence of one or more subterminal nerve

fibers innervating more than one muscle fiber (9, 20, 21, 22).

Coers et al. (21) observed a tendency of FTIR and ATIR to increase in humans with age. However, the correlation was not significant. Harriman et al. (49), however, observed axonal branching and elaborate and multiple end plates with elevation of terminal innervation ratios as an expression of aging in distal human leg muscles. This process apparently develops first and to the greatest extent at the distal end of the longest axons of the motor nerves.

Numerous other senile changes of nervous origin have been observed in aging animals. Gutmann et al. (43, 44) have cited such changes as decreases in RNA content in the motor nerves of the spinal cord, a decreased efficiency of nervous tissue to synthesize protein, and irregularities of cell and nucleus size.

Physiological properties of aging nervous tissue also demonstrate measurable changes. The physiological changes that occur within the neuromuscular apparatus have been a central focus of investigation because they tend to characterize age related changes in skeletal muscle. Frolkis et al. (34) have described these changes. Functional changes include increases in elecroexcitability thresholds and action potential time. Reductions are seen in membrane resistance, action potential amplitude, and lability. Metabolically, the concentration of intracellular

sodium increases while that of potassium decreases. These changes result in a decrease in the concentration differences between the cell and the extracellular space. The effect is seen most dramatically in the individual muscle fibers, where membrane permeability is changed (this alters the action potential as previously mentioned) and the sodium-potassium pump becomes modified in function. Frolkis et al. have drawn an interesting conclusion. They contend that the aging of skeletal muscle is a combination of age induced changes in the individual muscle cells, decreased neurotrophic function, and denervation. This combination is of such a nature that degenerative changes in the muscle fibers can lead to primary disruptions in motor nerve endings as well as neurogenic factors instigating muscle fiber degeneration.

Additional physiological evidence has been reported which demonstrates apparent neurogenic degeneration at or near the neuromuscular junction (16, 18, 42, 85). This includes reduced frequency of miniature end plate potentials (42, 43), reduced presynaptic axon conduction velocity (16), and synaptic depression in curare-blocked preparations (85).

Overview

An analysis of the literature reporting functional and structural changes in aging skeletal muscle led deVries (26)

to suggest three distinct contributing factors. Degenerative changes due to these aging phenomena may represent ony one contribution to the overall process. deVries cited disuse atrophy and unrecognized disease processes as two factors which may play a potentially dominant role in the degeneration of these functional properties. Of the three factors, deVries cited disuse atrophy as the only factor which may be realistically reversed.

Exercise And Senile Muscular Atrophy

Relatively few studies have been directed at determining whether or not exercise has any effect on the process of senile muscular atrophy. This may relate to a question of the trainability of organisms during senescence. Using skeletal muscle and organ weights as variables for determination of a training effect, McCafferty et al. (65) postulated the existence of a threshold age beyond which training has no effect on muscle and heart weights of geriatric rats. Initiation of training at 450 days resulted in minimal differences in muscle and organ weight between control and experimental groups. The same results were observed between experimental animals that began training at 600 days, and their control groups. It should be noted that the sample size for these groups was between 2 and 4 animals.

Taking another perspective, Drahota et al. (28) found that senile rats (24 months) were unable to initiate post-functional compensatory hypertrophy following experimental denervation. Younger rats, however, were able to initiate the process. The authors implied that the aforementioned may be an important consideration in utilizing exercise as a compensatory mechanism in senile muscular atrophy. The time period during which exercise is introduced may be crucial to its effectiveness. If exercise is introduced prior to the observable manifestations of functional denervation (about 24 months in the rat), it may have a more profound effect than if it is introduced after functional denervation has manifested itself.

Tomanek et al. (96) observed compensatory hypertrophy in the plantaris of 19 month old rats following denervation of the synergistic gastrocnemius. However, augmentation of elastin and collagen was observed in denervated control rats (45 days) but not in the old rats. The authors suggested that this may represent a substantial age associated decrement in the ability for adaptation.

Concerning the trainability of older humans (aged 70+), Andersen has concluded that improvements in the functional properties of the cardiovascular system require such intense training that it is questionable if the organism is trainable in senescence (2). Hollman (51) found no observable

improvement in the capacity for maximal and continuous effort in older individuals following training. The conclusion was made that virtually no improvement in functional capacity could be expected if training was initiated during the later stages of life.

Other studies, however, have reported that both older animals and humans substantially improved cardiovascular parameters and functional properties of skeletal muscle following regimented training programs (25, 26, 33, 37, 72, 77, 89).

Gutmann et al. (44) hypothesize that training, in the form of adaptation to repeated motor performance, may have a beneficial effect on the aging process. They cite the observation that the diaphragm shows the least amount of degeneration with age in relation to the total body musculature. They suggest that the constant "enforced" motor impulse activity associated with normal respiratory function might be visualized as continual exercise. They also postulate that this factor may be an important determinant in explaining the differing reaction of the diaphragm muscle.

Electrical stimulation of the lumbar spinal cord or the lumbar plexus following denervation has been shown to retard atrophy of denervated muscle fibers and to stimulate collateral sprouting in paretic rat muscles (30). When compared to untreated controls, the partially denervated muscles of the stimulated experimental animals showed 2.5

times the incidence of collateral sprouting. While these observations do not offer any direct evidence of an exercise effect in senile muscular atrophy, they do appear to support the notion of "enforced motor impulse activity" suggested by Gutmann et al. (44).

Jennekens et al. (54), in their autopsy study, found histochemical evidence of normal limb muscles in a 78 year old man who had led a very physically active life until his sudden death. The normalcy was with respect to fiber size. The sizes of the different fiber types in his muscles showed no atrophy in relation to male adult values. Type II fibers were larger than type I fibers. No conclusions were drawn from one case.

Retzlaff et al. (77) in a controlled longitudinal study of litter mate male rats, found marked structural and functional differences between those rats which had been exercised throughout their life span and those which had led a sedentary cage life. The exercised rats demonstrated enhanced spinal motor neuron integrative capacity, increased large fiber sciatic nerve conduction velocity and a slightly increased average life span.

Studies by Moritani and deVries (71, 72) have demonstrated that the training response (strength gain) in older individuals may not vary greatly from that of younger persons, if those responses are compared on a relative basis

(percentage gain). The authors found comparable increases in maximal voluntary strength between young (mean age 22 years) and old men (mean age 69.6 years), following eight weeks of isometric training (71). The similarity in strength gains became apparent when values were expressed as a percentage change in relation to initial strength values. The old subjects also demonstrated an increase in the level of maximal muscle activation in the immediate post-training period. This level was comparable to that of the young subjects.

Moritani and deVries also developed and utilized EMG instrumentation and methodology which enabled the separation of neural factors and hypertrophy involved in strength gain (24, 69, 70, 71, 72). The level of hypertrophy was determined by calculating the ratio of muscle activation (as mesured by IEMG) to the amount of force produced. The IEMG level attained during maximal voluntary contraction was the criteria for determining the contribution of neural factors. A maximal voluntary contraction represents maximal activation of the muscle involving maximal interaction of both facilitory and inhibitory factors not yet clearly understood (the "neural factors"). These neural factors may in turn indicate the integrity of the motor unit(s). Moritani and deVries utilized this technique to determine the relative contribution of "neural factors" and hypertrohy to strength gains in a group of young (18-26 years) and old

(67-72 years) men (70, 71). In the young group, neural factors accounted for the majority of the strength gain seen early in the progressive resistance training program. Hypertrophy then became the dominant factor after the first three to five weeks.

The old subjects demonstrated an absence of any muscle hypertrophy following the training regimen. The authors contended that strength gains in senescence can be expected following training. Additionally, increases in strength may entirely depend on neural factors with no observable hypertrophy.

Kiessling et al. (57) examined the effects of endurance training on the skeletal muscle of previously sedentary middle aged men (46-62 years). All subjects demonstrated an increase in maximal oxygen uptake of about eight per cent. The subjects showed only slight increases in cytochrome C oxidase activity and mitochondria volume. The authors interpreted these results as an indication of a general decrease in the organism's capacity of adaptation. However, it was noted that after training the subjects attained a maximal oxygen uptake approximately comparable to chronically well trained men of the same age. Although capacity for adaptation deceases with age, it is apparently not eliminated.

Similar results were observed by Suominen et al. (89)

in a group of 26 male and female 69 year old pensioners. Following an eight week aerobic training program, the mean maximal oxygen uptake of the males improved from 28.9 ml/kg/min to 32 ml/kg/min. The females showed a mean increase of 2.4 ml/kg/min from 27.9 to 31.3 ml/kg/min. Further indications of increased aerobic capacity were demonstrated by increased malate dehydrogenase activity in both groups. Additionally, the activity of lactate dehydrogenase decreased or remained the same in both groups. The authors noted that the activity of the two enzymes was higher in the male group both before and after training. Also the percentage of slow fibers in the muscle examined (m. vastus lateralis) was the same in both groups. deVries (25) reported increases in oxygen transport capacity and work capacity following 6-42 weeks of vigorous aerobic and anaerobic training. In addition, deVries found decreases in percentage body fat and systolic and diastolic blood pressure.

A general conclusion was drawn by many of the investigators reporting training responses in older animals and humans: Although the organism's capacity for adaptation decreases with age, it apparently is not eliminated (26, 33, 44, 72).

CHAPTER III

MATERIALS AND METHODS

Animals

Seventy colony bred Fischer-344 virgin female rats were used in the study. It was not possible to obtain all rats from only one source. However, all rats came from the same original breeder (Charles River Laboratories). One group of 30 animals was obtained directly from the Laboratories and the other was obtained from a colony maintained on campus. The animals were housed four to a cage (9 1/2 x 16 1/2 x 7) (Figure I) from birth, at a room temperature of 21.6° C and a relative humidity of 50%. Water and laboratory chow was supplied ad libitum.

All animals were 21 months of age at the onset of the investigation. The selection of animals in this age group was based on observations by Drahota (28), Tauchi (92), and McCafferty et al. (65). They found rats 20-21 months of age capable of postfunctional compensatory hypertrophy and devoid of any observable manifestations of senile muscular atrophy. In 24-25 month old rats, the same investigators noted that senile muscular changes were well manifested while no evidence of postfunctional compensatory hypertrophy was found. This evidence led McCafferty to suggest the existence of a threshold age beyond which measures, such as

Figure I. Animal Housing Facilities

training, have no effect on skeletal muscle. Based on the foregoing evidence, it is reasonable to assume 21 month old rats are on the threshold or just entering the observable stages of senile muscular atrophy and susceptible to training effects in skeletal muscle.

Training Protocol And Grouping Of Animals

Three groups of rats were established. Fifteen rats were sacrificed immediately and used as a baseline for comparisons with fifteen animals who were maintained under sedentary cage conditions as controls, and twenty animals placed in an experimental exercise group. Of the forty animals that started the training program, twenty were selected for the experimental groups. The others were eliminated because of injuries, poor running habits, or continual refusal to run.

The proposed exercise program was based on a modification of a program used by McCafferty (65). The protocol:

1) Training duration; five months. This brought the animals beyond the age where senile muscular changes are well documented (6, 92) to their mean life expectancy of 26.2 months (50).
2) Training modality; small animal treadmill (Quinton model 42-15) (Figure II).
3) Training frequency; five days per week.

Figure II. Motor Driven Exercise Treadmill

4) Target training period; thirty minutes per day.

5) Target training intensity; 23 meters per minute at an eight degree incline.

McCafferty was able to initiate increases in heart and kidney weight in adult rats exercised at 10-20 meters/minute for a period of five months. Although no data were found relating the target intensity to the percentage of maximal oxygen uptake in aged female Fischer rats, some estimations were made based on measurements of other young rat strains. Metabolic chamber studies on young adult male and female Sprague-Dawley, Wistar-Kyoto, and Okamoto-Aoki strains indicated that the work intensity of 23 meters/minute at an 8° incline represented a range of 65% to 75% of the experimental animals' maximal VO_2 (7, 13, 84). It was also suggested that any given work intensity represented a higher percentage of VO_2 max in older animals of the same sex and strain (7).

To encourage the animals to maintain the work intensity, mild electric shock was provided as incentive. Body weights were monitored as an indication of workload stress as well as subjective evaluation of each rat's ability to handle a given work load. Table I outlines the overall training protocol.

Table I

TRAINING PROTOCOL

Week	Time (min)	Incline (°)	Intensity (m/min)
One	5	4	8.2
Two	10	4	10.6
Three	15	4	10.6
Four	15	6	15
Five	20	6	15
Six	25	6	17.5
Seven	30	8	17.5
Eight	30	8	17.5
Nine	20	8	17.5
	10	8	20
Ten	20	8	17.5
	10	8	20
Eleven	15	8	17.5
	15	8	20
Twelve	10	8	17.5
	20	8	20
Thirteen	30	8	20
Fourteen	30	8	20
Fifteen	15	8	20
	15	8	23
Sixteen	30	8	23
Seventeen	30	8	23
Eighteen	30	8	23
Nineteen	30	8	23
Twenty	30	8	23
Twenty-one	30	8	23

Analytical Methods

Sampling Of Muscle

At the time of sacrifice, all animals were heavily anesthetized with ether and the soleus and gastrocnemius muscles from both hind limbs were exposed. All muscles were fixed at a standard length by extending the hind limb and freezing the gastrocnemius and soleus at that length with a freon probe. Both muscles were excised and thoroughly frozen in isopentane and liquid nitrogen.

The gastrocnemius and soleus muscles were selected for study because the rat utilizes both muscles during running. Because of this, they should have been actively stimulated by the training program.

The soleus has been widely utilized as a representative type I slow twitch fiber. Its chief advantages are its homogeneity (almost entirely type I fibers), its simple geometry, and its extensive tendon development, which simplifies mounting and extraction (19, 66). The gastrocnemius, on the other hand is a more mixed type muscle with a large percentage of type II fibers (19, 48, 66). However, in the present study, the percentage of type II fibers was much greater than the percentage of type I fibers. Although the geometry of the gastrocnemius is complicated (many fibers run obliquely to the long axis of the muscle), it offers the advantage of being easily removed

and it possesses an activity level which is very responsive to the exercise opportunities of the animal (48, 66).

Muscle and heart weights of all animals were taken for additional measurement of exercise or age related weight changes that occurred during the experimental period. The right soleus, right gastrocnemius, and heart were individually weighed. Also, the ratio of each tissue weight to total body weight was calculated. These measurements provided an index of both absolute and relative tissue weight changes.

Histochemical Techniques And Evaluation

Based on the background discussion of the published literature, the most popular theory on the primary mechanism of senile muscular atrophy is that the changes are neurogenic in nature (13, 32, 33, 34, 37, 38, 83). Because of the close relationship between the various properties of the motor neuron and the properties (structural, functional, and metabolic) and integrity of their corresponding fibers, neural degeneration will probably result in fiber atrophy and degeneration. For that reason, the following histochemical staining techniques were selected to demonstrate prominent aspects of this "neurogenic" degeneration.

I. Combined Silver and Acetylcholinesterase Stain

Beerman et al. have developed a combined acetylcho-

linesterase staining technique that clearly demonstrates the intramuscular innervation of fresh frozen tissue (8). The authors have demonstrated that this procedure exhibits good morphology of denervation-invoked degeneration of neuromuscular innervation as well as nerve terminal sprouting. This method is described in detail by the authors (Appendix Table I) and has been selected for its ability to demonstrate end plate degeneration and adaptation. The method simultaneously stains intramuscular nerves, subterminal axons and motor end plates either black or brown. There is minimal staining of related connective tissue and muscle fibers. Longitudinal frozen sections 30-100 microns thick will be taken from the midsection of the muscle.

Evaluation

Besides descriptive evaluation of end plate degenerations observed by Gutmann (38), and end plate elaboration (growth configurations) associated with denervation described by Tuffery (99), a mathematical evaluation among experimental and control groups was made. This evaluation was made according to a procedure used by Beerman (8), Beerman et al. (9), and Coers et al. (21) for determining functional and absolute innervation ratios. The ratios can be calculated according to the following equations:

$$\text{FTIR} = \frac{\text{The number of muscle fibers innervated by a given number of subterminal axons}}{\text{The number of subterminal axons}}$$

$$\text{ATIR} = \frac{\text{The number of motor end plates innervated by a given number of subterminal axons}}{\text{The number of subterminal axons}}$$

Counting of axons, muscle fibers and motor end plates was done according to a modification of a number of arbitrary rules utilized by Coers et al. (21), Beerman et al. (8), and Beerman (9). Axons which terminated at 1 motor end plate or 1 muscle fiber were assessed as 1:1 for FTIR and ATIR. Double end plates on a single muscle were assessed as 2:1 for ATIR and 1:1 for FTIR. Axons had to measure 50 micrometers or more to be counted if they could not be traced to an intramuscular nerve. Also, only axons which terminated at motor end plates were counted.

To evaluate the incidence of growth configurations, counts of collateral sprouts and collateral contributions were made. Pilot work had shown that there was great difficulty in accurately determining between sprouts and contributions in rat muscle. Thus, collateral sprouts and contributions were classified together as growth configurations (nerve terminal sprouting) which in turn were expressed as a ratio or percentage of the total number of subterminal axons per sample. This allowed for statistical

comparisons between groups.

II. Myosin ATPase Stain

Staining for myofibrillar adenosine triphosphate (ATPase) activity clearly elucidates the differences between type I and type II fibers when the sections are preincubated in the proper pH medium (12, 74, 87, 90). Differentiation of small, angulated fibers is also easily accomplished utilizing this type of stain (15). In the present study, a myosin ATPase procedure developed by Suzuki et al. (90) was employed (Appendix, Table II).

Frozen sections 8-10 microns thick were cut at -20° C and duplicate samples were stained for ATPase activity with acid (pH 4.3) and alkaline (pH 10.3) preincubation. Type I fibers demonstrated a low ATPase activity following alkaline preincubation (10.3) and a high ATPase activity following acid preincubation (4.3) (31, 90). Type II fibers showed the opposite staining characteristics. Additionally, type II fibers preincubated at the alkaline pH, demonstrated a group of dark staining fibers and one of intermediate staining intensity. Both groups were classified as type II fibers. Because of a reversal of staining characteristics with alkaline and acid preincubations, more accurate classification of fiber types were possible when using contrasting preincubations.

Evaluation Of Small Diameter Fibers

To elaborate group differences in the number of small diameter fibers, a method utilized by Adams (1) was chosen. This involved determination of the ratio of small fibers to the total number of fibers in cross section. The result was a ratio or percentage which could be used statistically to compare experimental and control groups. Using an eyepeice micrometer, cross sections were divided into four quadrants. A minimum of 100 fibers per quadrant were counted for determination of the percentage of small diameter fibers. The soleus cross sections, which had many fewer total fibers than the gastrocnemius cross sections, were counted in their entirety.

As an additional measure of control, an arbitrary maximum fiber diameter was chosen as a criterion for classification of small diameter fibers. This maximum diameter was set at two standard deviations below the mean fiber diameter of the 21 month old baseline group. Any fibers with a diameter greater than this criterion distance were classified as "normal" fibers.

An arbitrary sequence of classifying small diameter fibers was followed. Potential small diameter fibers were first selected on the basis of their angulated, abnormal geometrical shape. They then had to meet the size criteria for final determination.

Evaluation of Fiber Types And Fiber Diameters

Fiber type percentages were determined in the same manner as the percentage of small diameter fibers. The cross sections were divided into quadrants and a minimum of 100 fibers per quadrant were classified as type I or type II fibers, based on their myosin ATPase staining characteristics. Counts were as random as possible and grouping of fiber types was included in the calculations. Based on this representative sampling, the percentage of type I and type II fibers were computed for each muscle.

Fiber diameters were measured using an eyepiece micrometer. The fiber diameter was determined as the lesser diameter of each fiber. A minimum of 100 random type I and 100 random type II fibers were measured in each muscle sample. In some cases, 100 fibers of a given type were not present. In this instance all diameters of that particular fiber type were measured. Fiber diameter ratios were expressed as the ratio of the mean type I fiber diameter to the mean type II fiber diameter for each muscle sample.

Analysis of Data

To initially determine if any significant differences among groups were present, a one way analysis of variance was employed (32). When a critical F value was calculated at either the .01 or .05 level, then an a posteriori test was performed. The Student-Newman-Keuls test, for multiple

comparisons among means based on unequal sample sizes, was utilized in these cases (86).

CHAPTER IV

RESULTS

Mean Body Weight, Heart Weight, and Heart Weight/Body Weight Ratio

Mean body weights, absolute heart weights, and relative heart weights (heart weight/body weight ratio) are presented in Table II. There was no significant difference in mean body weight between the baseline and control group. The exercise group weighed significantly less than either of the other two groups ($p<0.01$). The exercise group also displayed a mean weight loss of 8.2 grams from pre-training to immediate post-training conditions. This, however, was not statistically significant.

No significant difference was noted in absolute, uncorrected heart weights, but when a cardiac index was calculated, i.e. heart weight/body weight, the mean exercise index was significantly larger than the other two groups ($p<0.01$). The cardiac index is a indicator of relative cardiac hypertrophy.

Gastrocnemius and Soleus Weight and Muscle/Body Weight Ratio

Mean weights for the right gastrocnemius and soleus

muscles, as well as the mean ratios of each muscle weight to total body weight are shown for all three groups in Table III. No statistical differences among groups were observed for right gastrocnemius weight. The mean right soleus weight of the exercise group was found to be significantly less than either the baseline or control weights ($p<0.01$). No statistical difference was observed between the baseline and control soleus weight.

When expressed as a ratio of right muscle weight to total body weight, the gastrocnemius exercise group had a significantly larger ratio than either the baseline or control groups ($p<0.01$). No significant difference was demonstrated between the baseline and control groups. No significant differences were found among groups for the soleus ratios.

Fiber Dimensions and Type I/Type II Fiber Diameter Ratios

Table IV presents the mean type I and type II fiber diameters, as well as the mean type I/type II fiber diameter ratios, for both muscles. The only significant difference observed was between the soleus exercise type II group and the control type II group ($p<0.01$) and between the soleus exercise type II and the baseline type II groups ($p<0.01$).

The only significant differences regarding the type I/type II fiber diameter ratios were found between the

gastrocnemius exercise and control groups, the soleus exercise and baseline groups, and the soleus exercise and control groups. All differences were significant at the 0.01 level.

Percentage of Small Diameter Fibers

Table V displays the results of small diameter fiber counts for all groups. The incidence of small diameter fibers was expressed as a percentage of total fibers counted. The gastrocnemius demonstrated significant differences among all three groups. The control group exhibited a larger percentage than either the exercise or baseline groups ($p<0.01$). The exercise group had a larger percentage of small diameter fibers than the baseline group ($p<0.01$) but a smaller percentage than the control group ($p<0.05$). Tabulations from the soleus muscles revealed that the control group and exercise group had significantly larger percentages of small diameter fibers than the baseline group ($p<0.01$).

When viewed under the light microscope, sections from all groups of both muscles demonstrated many similarities. All cross sections exhibited predominately normal, regularly sized muscle fibers. Scattered among these normal fibers were angulated, small diameter fibers. They usually appeared singularly, but occasionally 2 to 4 of these fibers

were grouped together. These fibers took on various abnormal geometric shapes. Many were elongated and thin, while others were rectangular, polygonal, or even concave. On occasion one or two thin fibers could be seen curved or wrapped around the border of a large normal fiber. Another characteristic present in all groups was the observation of two small fibers situated in a book end configuration. Plate I illustrates examples of the above mentioned patterns.

Fiber Type Percentages

Mean soleus and gastrocnemius fiber percentages are presented in Table VI.

The gastrocnemius showed no significant differences in fiber type percentage among groups. A distinct, consistant arrangement of fiber types was seen in the three groups. The majority of the type I fibers seen in cross section were located in a circular area near the center of the muscle. The rest of these fibers were distributed randomly throughout the muscle. Occassionally, two or three type I fibers were grouped together on the perimeter.

The soleus showed a changing pattern. The percentage of type I fibers in the exercise group was significantly greater than that of the baseline and control groups ($p<0.01$) additionally, there appeared to be differences in

fiber pattern as seen in cross section. The baseline group demonstrated a fairly homogeneous mixing of type I and type II fibers. The control and exercise groups also displayed this mixing, but to a lesser degree. Type II fiber grouping was apparent in the control and exercise groups while not readily observable in the baseline group.

Growth Configurations and Terminal Innervation Ratios

Shown in Table VII are the observed percentages of growth configurations and the mean and pooled FTIR's and ATIR's for each group. The mean ratios were calculated from the TIR's for each muscle, while the pooled TIR's were calculated by combining all the data in a given group.

The only significant differences for the gastrocnemius were observed with respect to the ATIR's. The exercise group was found to be greater than both the control group ($p<0.05$) and the baseline group ($p<0.01$). No differences were found for the FTIR's. The soleus displayed no significant differences in ATIR's or FTIR's among groups. The exercise group demonstrated a higher incidence of growth configuration than either the control or baseline groups ($p<0.01$). The control group also showed a significantly higher incidence of these growth configurations than the baseline group ($p<0.05$).

Microscopic observation of the motor end plates revealed a number of characteristic morphological changes.

The changes were observed primarily in those end plates demonstrating growth configurations. These end plates often displayed what appeared to be multiple contributions. These nerve terminal branchings were often so complex that it was difficult to classify them morphologically. Many of these branchings appeared to be ultraterminal sprouts. Others appeared to be more complex extensions of simple tree-like and ring-like forms of the terminal ramification of the synaptic apparatus. Often these end plates were elongated and thickened. Apparent vesicular axonic swelling was occasionally observed as well as what seemed to be an unfolding of the subneural apparatus. Sometimes an end plate was observed that appeared to be fragmented. These fragmented pieces were usually connected by branchings from the subterminal axon or from branchings between the fragments. Plate II illustrates various examples of this end plate sophistication.

Plate I. Photomicrographs of Isolated Small Diameter Fibers:

(A) Control Gastrocnemius, pH 10.4, 400x;
(B) Baseline Gastrocnemius, pH 10.4, 400x;
(C) Control Gastrocnemius, pH 10.4, 400x

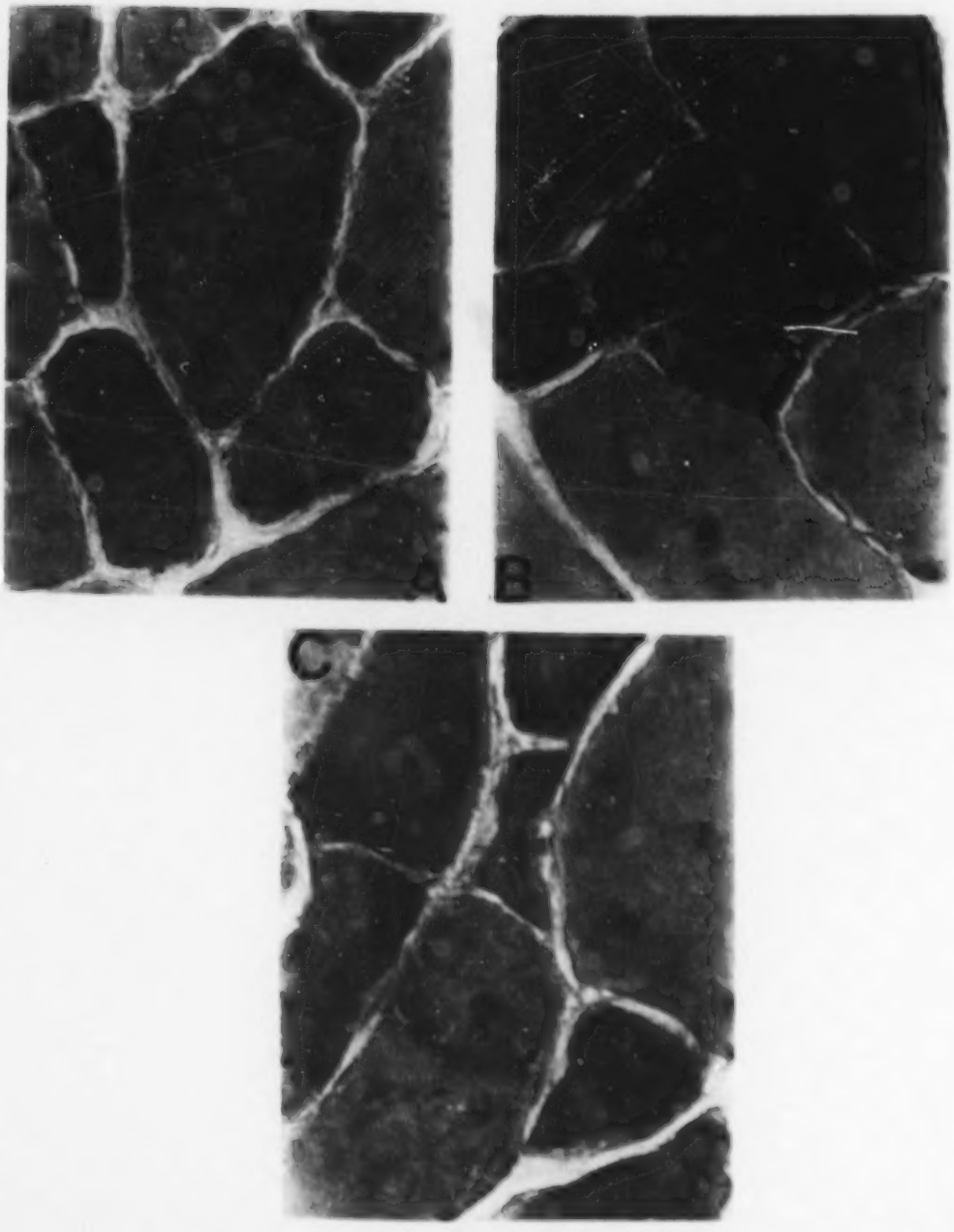
A
B
C

Plate II. Photomicrographs Depicting Various Examples of Endplate Sophistication and Nerve Terminal Branching:

(A) Exercise Soleus, 400x;
(B) Control Soleus, 400x;
(C) Exercise Soleus, 400x;
(D) Baseline Soleus, 400x

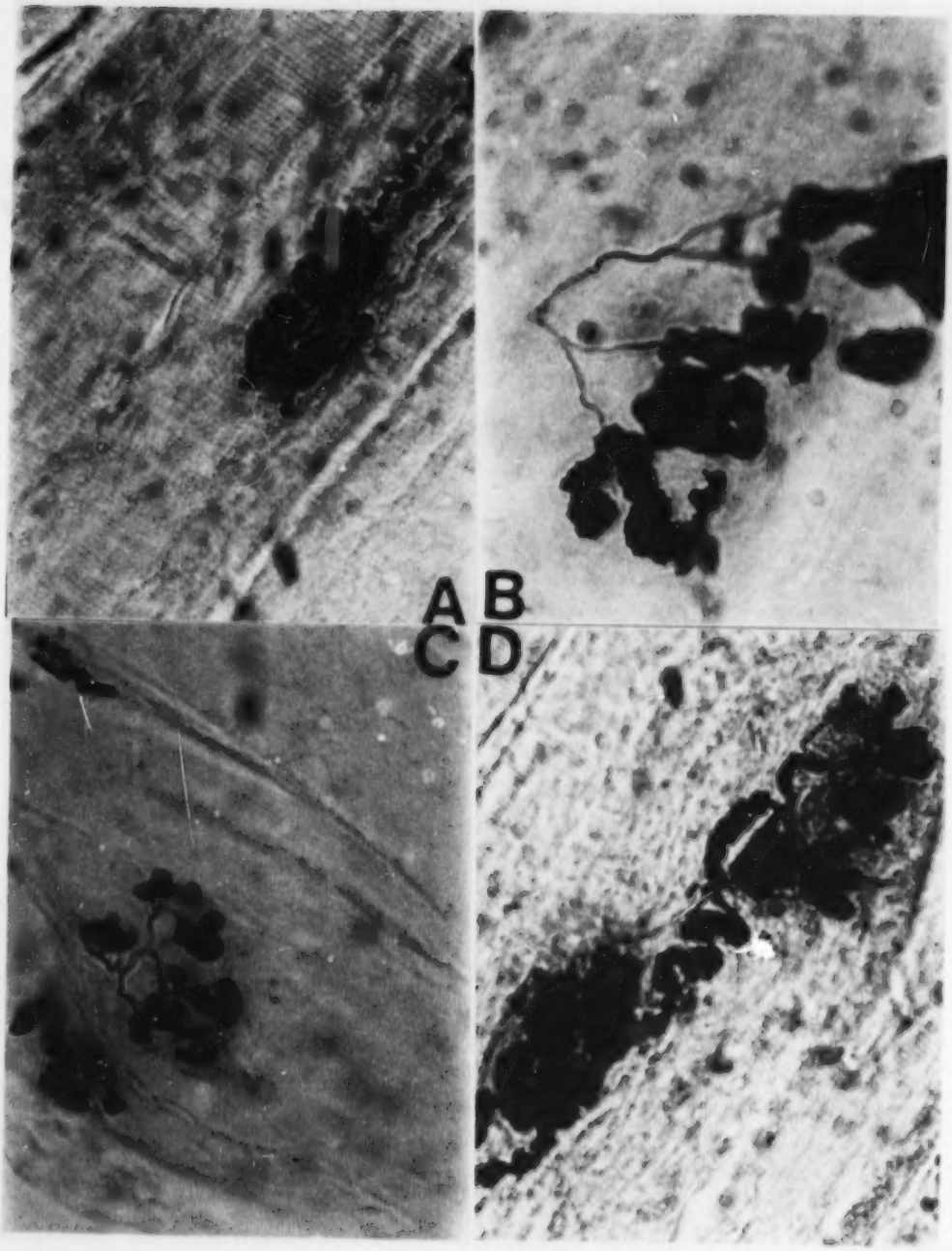
A
B
C
D

Table II

MEAN BODY WEIGHT, HEART WEIGHT, AND HEART WEIGHT/BODY WEIGHT RATIO

		Body Weight (gm)	Heart Weight (gm)	Ratio (HW/BW)
Baseline	$\bar{X}$	263.2	0.94	0.0036
N = 15*, 13**	S.D.	34.5	0.15	0.0006
Control	$\bar{X}$	276.7	0.94	0.0035
N = 15*, 14**	S.D.	34.9	0.09	0.0005
Exercise	$\bar{X}$	219.5[c,d]	0.99	0.0045[a,b]
N = 20*, 15**	S.D.	8.6	0.10	0.0005
Pre-Exercise	$\bar{X}$	227.7[c,d]	—	—
N = 20	S.D.	11.4	—	—

*N for body weights
**N for heart weights and heart weight/body weight ratios

[a]Significantly greater than the baseline group ($p<0.01$)
[b]Significantly greater than the control group ($p<0.01$)
[c]Significantly less than the baseline group ($p<0.01$)
[d]Significantly less than the control group ($p<0.01$)

Table III

MEAN GASTROCNEMIUS WEIGHT, MEAN SOLEUS WEIGHT, AND RATIO OF GASTROCNEMIUS AND SOLEUS WEIGHT TO BODY WEIGHT

		Gastrocnemius		Soleus	
		Right Muscle Weight (gm)	Right Cas. Wt. / Bd. Wt.	Right Muscle Weight (gm)	Right Sol. Wt. / Bd. Wt.
Baseline	$\bar{X}$	1.08309	0.0042	0.07971	0.000305
N = 15	S.D.	0.09890	0.00036	0.01133	0.000044
Control	$\bar{X}$	1.09158	0.0040	0.08099	0.000295
N = 15	S.D.	0.11327	0.00039	0.01278	0.000053
Exercise	$\bar{X}$	1.03005	0.0047[a,b]	0.06675[a,b]	0.000304
N = 20	S.D.	0.04562	0.00021	0.00413	0.000019

[a]Significantly different from the baseline group ($p<0.01$)
[b]Significantly different from the control group ($p<0.01$)

Table IV

TYPE I AND TYPE II FIBER DIAMETERS AND TYPE I/TYPE II FIBER DIAMETER RATIOS

		Gastrocnemius			Soleus		
		Type I Diameter (μm)	Type II Diameter (μm)	Ratio Type I/II	Type I Diameter (μm)	Type II Diameter (μm)	Ratio Type I/II
Baseline	$\bar{x}$	35.81	43.86	0.81	47.10	40.83	1.18
N = 10	S.D.	5.08	3.70	0.08	3.71	6.97	0.14
Control	$\bar{x}$	32.64	44.17	0.74	46.05	40.12	1.15
N = 12	S.D.	4.47	3.00	0.12	2.91	2.13	0.07
Exercise	$\bar{x}$	32.83	46.36	0.71[a]	45.14	30.45[a,b]	1.61[a,b]
N = 16	S.D.	3.50	2.52	0.08	3.17	3.23	0.54

[a]Significantly different from the baseline group ($p<0.01$)
[b]Significantly different from the control group ($p<0.01$)

Table V

MEAN PERCENTAGE OF SMALL DIAMETER FIBERS

		Gastrocnemius	Soleus
Baseline	$\bar{x}$	9.8	6.3
N = 10	S.D.	2.8	3.1
Control	$\bar{x}$	16.0[a]	9.8[a]
N = 11	S.D.	3.7	2.2
Exercise	$\bar{x}$	13.4[a,b]	8.8[a]
N = 16	S.D.	2.4	1.5

[a]Significantly different from the baseline group ($p<0.01$)
[b]Significantly different from the control group ($p<0.05$)

Table VI

MEAN FIBER TYPE PERCENTAGES

		Gastrocnemius		Soleus	
		Type I	Type II	Type I	Type II
Baseline	$\bar{x}$	9.7	90.3	80.9	19.1
N = 10	S.D.	5.6	5.6	5.8	5.8
Control	$\bar{x}$	9.0	91.0	84.1	15.9
N = 11*, 12**	S.D.	1.7	1.7	5.2	5.2
Exercise	$\bar{x}$	9.3	90.7	92.0[a,b]	8.0[a,b]
N = 15	S.D.	2.4	2.4	3.8	3.8

*Gastrocnemius
**Soleus

[a]Significantly different from the baseline group ($p<0.01$)
[b]Significantly different from the control group ($p<0.01$)

Table VII

MEAN PERCENTAGE OF GROWTH CONFIGURATIONS AND MEAN AND POOLED FTIR'S AND ATIR'S

		Gastrocnemius					Soleus				
			FTIR		ATIR			FTIR		ATIR	
		% Growth	Mean	Pooled	Mean	Pooled	% Growth	Mean	Pooled	Mean	Pooled
Baseline	X	21.3	1.05	1.05	1.08	1.08	30.4	1.07	1.06	1.10	1.10
N = 10*, 8**	S.D.	6.0	0.03	—	0.02	—	3.0	0.02	—	0.01	—
Control	X	28.4	1.06	1.06	1.10	1.11	37.5[c]	1.06	1.06	1.10	1.12
N = 14*, 15**	S.D.	10.4	0.02	—	0.02	—	6.0	0.03	—	0.04	—
Exercise	X	27.6	1.06	1.06	1.14[a,d]	1.14	47.3[a,b]	1.06	1.06	1.12	1.13
N = 16	S.D.	6.6	0.02	—	0.06	—	9.6	0.02	—	0.03	—

*Gastrocnemius
**Soleus

[a]Significantly different from the baseline group ($p<0.01$)
[b]Significantly different from the control group ($p<0.01$)
[c]Significantly different from the baseline group ($p<0.05$)
[d]Significantly different from the control group ($p<0.05$)

CHAPTER V

DISCUSSION

Body Weights and Heart Weights

The exercised rats in the present study showed a slight reduction in mean body weight following the five month training program. This reduction was compatible with results from other training studies on rats (23, 57, 76, 88). This slight decrease in body weight was probably related to two factors. First was a decreased food intake. Stevenson et al. (88) found that prolonged exercise acts as a mild appetite depressant in rats resulting in voluntary reduction in caloric intake. Second was an increased caloric expenditure. Crews et al. (23) have found that 9-18 weeks of treadmill exercise resulted in a marked decrease in the fat content of exercised rats relative to sedentary controls.

It was possible that a loss of lean body weight contributed to the overall weight loss in the exercise group. This appears unlikely. There was little difference in heart and gastrocnemius weight among groups. When expressed as a percentage of total body weight, the percentages for heart, gastrocnemius, and soleus of the exercise group were the same as or larger than those of the other two groups. This suggests that the amount of lean

body weight in the exercise group was greater than or equal to that of both the control and baseline groups.

It was not possible to make any matched pair comparisons between either the baseline and exercise groups or the control and the exercise groups. The reason for this was that both the baseline and control groups were significantly heavier than the exercise group prior to training. To secure the number of rats needed for the study, animals were obtained from two sources. However, all animals were originally obtained from the same breeder (Charles River Laboratories, MA). Normally groups could be mixed to compensate for differences in weight resulting from differences in litter size and cage population. It was decided not to mix the animals among groups for fear of introducing infection from one source to another. Older rats are quite susceptible to respiratory infections and these infections are easily spread when mixing rat colonies. The weight difference will be noted when discussing differences among groups that may be function of body weight.

No significant difference in mean body weight between the baseline and control groups was observed. In fact, the control group was slightly larger (13.5 grams). Other studies have reported a loss in the body weight of old rats as they approached their mean life expectancy (44, 57).

However, these observations were made with respect to male rats. Female rats were utilized in the present study. Yiengst et al. (103), have observed a weight loss in male rats from 18 months of age to 24 months. However, they also found an increase in the weight of female rats during the same time period. The authors suggest that, since the longevity of the female rat is greater than that of the male, a different pattern of weight gain and loss is involved in senescence. This may explain the absence of a weight loss in the control group in the present study.

The finding of no significant differences in heart weights among groups in the present study was contrary to the results of McCafferty et al. (57). They found smaller heart weights in their older exercised animals when compared to a control group. It appears from the present results that the exercise program did not have the negative effect on heart weight that McCafferty et al. found. This was observed even though the current training program utilized a greater intensity.

However, a difference among groups in the present study was apparent when the heart weights were expressed as a percentage of body weight (cardiac index). The significantly greater mean heart weight/body weight ratio of the exercise group relative to the other two groups demonstrates an apparent relative myocardial hypertrophy following training, especially since the mean body weight was not

significantly different from the pretraining weight. This has previously been demonstrated in younger rats (76, 100). Peterson (76) observed this relative hypertrophy in the absence of increased heart weight. He established that this relative hypertrophy was a result of various alterations in anatomical conponents of the heart such as mean fiber diameter and capillary/fiber ratio. Thus, the existence of relative hypertrophy may be offered as indirect evidence of a training effect in the present study.

Muscle Weights, Fiber Diameters, and Type I/Type II Fiber Diameter Ratios

The lack of a significant difference in body weight between the baseline and control groups was paralleled by an absence of differences in soleus and gastrocnemius weight respectively. The fact that there was no change in the percentage of right gastrocnemius and right soleus mass to body weight, suggests that there was also no real change in body composition. This differs from the findings of other investigators who have observed a decrease in muscle mass during this time period (57, 103). Often this decrease exceeded the loss of total body weight as illustrated by a reduced ratio of muscle mass to body weight (103). As was the case with body weight, the muscle weight values were obtained from male rats. When comparing muscle weight of 18

and 24 month old female rats, Yiengst et al. (103) found no significant differences in muscle mass and a slight decrease in the percentage of muscle mass to body weight. This agrees with the data from the female rats in the present study. The slight decrease in the percentage of right soleus and gastrocnemius mass to total body weight was not significant. However, the calculated least significant range (LSR), from the Student-Newman-Keuls test, was very close to the critical value needed for significance at the 0.05 level.

The gastrocnemius weight of the exercised rats was not statistically different from that of the baseline or control groups. The fact that the exercised soleus weight was significantly less than the other two groups suggests a differential response to the training program. One possible explanation is that the soleus is less adaptable to a given load in senescence. However, considering the work intensity of the training program (60-80% VO_2 max)and the vast difference in fiber pattern between the soleus and gastrocnemius, it seems likely that the difference in response of the two muscles was due to differences in fiber recruitment. At work intensities of 60-80% of maximal VO_2, the predominant fiber type recruited is the Type I (slow-oxidative) (29, 82). The soleus muscle in the present study exhibited predominantly type I fibers (greater than 80% for all groups) while the gastrocnemius displayed predominantly

type II fibers (greater than 90% for all groups). These facts suggest that the soleus was recruited to a far greater extent during training than was the gastrocnemius. Since both muscles were probably not utilized to the same degree during exercise, it follows that they would not demonstrate the same degree of weight change.

Although the mean soleus weight was least in the exercise group, the ratio of right soleus weight to total body weight (SW/BW) was not different among groups. For the gastrocnemius, this ratio (GW/BW) was significantly larger for the exercise group when compared to the baseline and control groups.

The ratio of muscle weight to body weight is an indication of relative lean body weight. The fact that the exercise (SW/BW) and (GW/BW) were greater than or equal to those of the other two groups, suggests that the training program did not have the profound effect on lean body weight hypothesized by McCafferty (57). It is possible that the much lower initial and final body weights for the exercise group may have confounded these ratios.

The question of muscle size in the exercise group prior to training is also an important consideration in any comparisons among groups. If it is assumed that the gastrocnemius did not hypertrophy as a result of exercise (there were no differences in fiber diameters among groups)

then the hypothesis that the soleus weight of the exercise group was approximately equal to that of the baseline group prior to exercise must also be assumed. Based on that assumption, a mean decrease in right soleus weight of about 16% occurred following training. This effect of exercise on soleus weight suggests a greater involvement of the muscle during the training program. Whether or not this indicates a degenerative effect on the muscle will be discussed later, in relation to other structure parameters.

The fiber diameter data offers additional information which may help explain the post training weights of the gastrocnemius and soleus. The fact that the gastrocnemius exhibited no significant differences in weight among groups was also reflected by a lack of difference in type I and type II fiber diameters. This pattern was also displayed by the soleus baseline and control groups. The significantly lighter soleus exercise group showed no difference in mean type I diameter, but a considerably smaller mean type II diameter when compared to the other two groups.

To account for the 16-17% difference in the exercise soleus weight entirely in terms of a type II fiber atrophy is not practical. This may be illustrated by an example. Although the exercise type II fibers were, on the average smaller than those of the control group, they represented less than 10% of the total fibers seen in cross section. If it is assumed that the type II fibers in the control group

weighed approximately the same as the type I fibers, a 25% reduction in their weight (10-15% of the total muscle weight) would result in a overall weight reduction of only about 2-4%. Additionally, the weight difference cannot be accounted for in terms of fiber drop out either, since the incidence of degenerating, necrotic fibers was not significantly different between the control and exercise groups. There is the possibility that a large age induced fiber drop out could have occurred prior to the completion of the training program. However, the normal appearance of the exercise group type I fibers along with a lack of any substantial degeneration in the exercise group suggest that this is not a viable explanation.

It is not readily apparent why the exercise group was lighter than the other groups. One possible explanation for this weight difference might be attributed to differences in intramuscular fat deposition. This could only account for a small portion of the difference, however, since fat deposits in sedentary old rats (24 mos) have been reported to represent less than 5% of overall muscle weight (103).

Another contributing factor may have been related to differences in soleus extraction among groups. When removing the soleus from the exercised animals, the tendon attachments were severed proximally first, and then distally. This resulted in some difficulty in excising the

complete tendon at the base of the attachment. The sequence was reversed during the removal of the baseline and control muscles, yielding a more complete excision at both attachments. The difference in the amount of tendon removed may have been reflected in a corresponding difference in soleus weight. This is particularly important with respect to the Achilles tendon. It is a thick, dense tendon, and its junction with the soleus is often surrounded by lipid deposits, potentially adding to its weight (58).

Another problem in removing the exercise group muscles was the difficulty in separating it from other muscles and from the bone. A greater length of the tendon was attached to the bone and the connective tissue holding muscle groups together was more tightly bound. This too may have interferred with the complete removal of the tendons in the exercise group. The source of this increased connective tissue adherence was not examined. Possibly a greater amount of collagen crosslinking was stimulated during training.

Type II fiber diameters demonstrated another area of measurement where a difference between the exercise and control groups was found. The smaller type II fiber diameters in the exercise soleus may be related, at least in part, to the form of training. Endurance training, as in the nature of the present study, has been shown to result in small deceases in the type II fiber areas of primarily slow

twitch endurance muscle (82, 93). This is believed to be a consequence of changes in fiber type recruitment pattern. However, the decrease seen in the present study is greater than reported for young mature adults and suggests that the organism may be more susceptible to this effect during aging. Whether the smaller exercise group type II mean diameter represents a negative effect brought about by the exercise program, one which can be correlated with a specific decline in soleus function, cannot be established on the basis of the present results.

The type I/type II fiber diameter ratios represent a measurement of relative atrophy. The lack of any significant difference between the baseline and control groups for either muscle indicates a lack of any relative atrophy. No difference was found between the exercise and control groups' ratios. This suggests no exercise effect occurred in this muscle. The significant difference in fiber diameter ratios between the gastrocnemius baseline group and the exercise group is apparently due to the slightly lesser type I fiber diameter and the slightly larger type II fiber diameter in the exercise group. The differences between the exercise soleus and the baseline soleus and between the exercise soleus and the control soleus appear to be primarily related to the much smaller exercise mean type II fiber diameter.

The lack of substantial age changes in muscle weight and fiber diameter make it difficult to draw any conclusions as to the possible effect of exercise on these parameters. It appears that these changes do not manifest themselves until much later in the life span of the female rat. However, it appears that exercise, initiated late in the life of the female rat, does not have a deleterious effect on fiber dimensions with resulting structural degeneration.

Small Diameter Fibers

Results of the incidence of small diameter fibers suggests that an aging and an exercise effect occurred. The apparent aging effect is illustrated by the significantly greater mean percentages of small diameter fibers observed in the gastrocnemius and soleus control groups when compared to their corresponding baseline values. The fact that the gastrocnemius exercise group demonstrated a smaller percentage of small diameter fibers than its control group offers evidence for an exercise effect. The soleus control group had a slightly larger percentage of small diameter fibers than the soleus exercise group. Although this difference was not significant, the calculated LSR from the Student-Newman-Kuels test was very close to the critical value for significance at the 0.05 level.

An important consideration concerning the small

diameter fibers is the question of their fiber type. Do these small diameter fibers represent a random contribution of both type I and type II fibers or is one fiber type dominant? A random contribution would be characterized by small diameter fiber type percentages that reflect the fiber type of the entire cross section of muscle. For example, assume a particular muscle was found to have 90% type I fibers and 10% type II fibers. To establish a random contribution from both fiber types to the small diameter population, the fiber typing of the small diameter fibers would require a resulting count of approximately 90% type I and 10% type II fibers.

To check for randomization or dominance in the present study, the small diameter fibers of five animals from each group were fiber typed. This was done for both muscles. In every case, the fiber type percentages of the small diameter fibers paralleled those of the entire muscle. As previously discussed, this indicates that the small diameter fibers represent a random contribution from both fibers types.

Fugisawa (35) and Tomlinson et al. (97) have utilized a grading system which classifies the incidence of small diameter, atrophied fibers seen in aging and muscular disease, by degree of severity. These changes have been graded into four categories of increasing atrophy.

Grade I atrophy represents minimally affected muscle fibers. Grade I sections, when compared to healthy adult

sections, demonstrate primarily normal, regularly-sized muscle fibers. The presence of single small, angulated fibers or groups of 3 to 5 very small fibers are occassionally observed. These small fibers are frequently elongated thin and polygonal. This sets them apart geometrically from the more normal fibers.

Grade II atrophy describes sections in which the majority of the fibers are still normal. However, there is a greater variation in fiber diameter and a more frequent, larger grouping of small diameter fibers.

Grade III atrophy is characterized by sections in which there is a very striking variation in fiber size and the sections exhibit as many small fibers as normal fibers.

Grade IV atrophy classifies sections where the great majority of the fibers are small.

When applying this grading system to the present study, all sections met the criteria for either grade I or grade II atrophy, with the majority of the sections fitting the description for grade I atrophy.

Fugisawa (35) and others (24, 54, 97, 98) have reported age changes in human and rat muscle in which the great majority of fibers were small (grade IV atrophy). In the present study the gastrocnemius control group exhibited the highest incidence of small fibers, which was only sixteen percent. No sections meeting the criteria for more severe

atrophy (grade III or IV) could be found. This low incidence of degenerating fibers adds to the previous findings indicating that only a minimal amount of age related fiber degeneration had occurred in the present study.

The significant difference in small fiber percentages between the gastrocnemius exercise and control groups suggests that fiber atrophy may be reduced somewhat by exercise. Although the practical significance of this small difference (2.6%) is suspect, it seems reasonable to assume that with a greater age related degeneration, this exercise effect might become more profound. This probability is enhanced by the fact that the exercise effect was observed in association with such minimal morphological evidence of atrophy.

The lack of a significant difference between the exercise and control soleus groups may be related to the overall low incidence of small fibers observed in all these groups. A marked increase in the incidence of small fibers may be required before any practical comparisons between sedentary and exercise muscle can be made. It appears that the soleus is more resistant to fiber atrophy than the gastrocnemius in terms of the incidence of small diameter fibers.

Fiber Type Percentages

Changes in the percentage of type I and type II fibers during aging have been attributed to two factors. One is the preferential degeneration and corresponding decrease in the number of one fiber type (6, 95). The other is a possible conversion of one fiber type to another (15).

Interpretation of changes in fiber type following training are dependent on the type of histochemical stain which is utilized. When using histochemical staining for mitochondrial enzymes, such as nicotinamide adenine dinucleotide diaphorase or succinate dehydrogenase, an increase in red fibers (type I) is usually observed following endurance training (5, 29, 82). However, this is generally accepted as a function of an increased activity of the mitochondrial enzymes of both type I and II fibers rather than a conversion of type II to type I fibers (82).

No change in fiber type percentages has been reported following training when the proportion of type I and type II fibers are determined according to the histochemical activity of myosin ATPase (5, 29, 82). Myosin ATPase staining is related to the contractile properties of the individual fibers. The histochemical ATPase activity of given fiber is believed to be a result of the concentration of myosin or of the integrity of myosin or of myosin ATPase in the myofibrils (5, 29).

Until recently, conversion of fiber types, as determined by myosin ATPase staining, could only be observed following drastic invasive techniques such as cross-innervation. However, recent findings have indicated that conversion may be possible through other mechanisms, such as hormonal stimulation. For example, a thyroidal trophic influence has been reported (52). Thus, the potential for fiber type conversion during aging may be a plausible development.

In the present study, differences in fiber type percentages among groups were present only in the soleus. The gastrocnemius displayed almost identical proportions of type I and type II fibers among all three groups. The differences between the two muscles may be related to differences in their dynamic properties. Kugelberg (60) has suggested that during development in the rat, adaptive changes take place within skeletal muscle that balance speed of movement with economy of energy. He contends that with an increasing leg length, rapidity of leg movement will be reduced. This correlates with a diminished need for fast motor units. With increasing body weight, a postural muscle like the soleus, will have an increased need for slower motor units capable of inducing tonic contractions. This results in a redistribution of the muscle to the growth of the animal.

Kugelburg has also suggested that chronic changes in

workload, through out the lifespan of the rat, may contribute to changes in muscle fiber pattern. Both long term increases and decreases in activity pattern may contribute to these changes. Postural muscles exhibit the greatest adaptive capability in this respect, particularly the soleus (17, 60).

Although the difference between the soleus baseline and control groups in percentage of type I fibers was not significant, (control was greater than baseline), the calculated LSR was again very close to the critical value for significance at the 0.05 level. This suggests a trend that agrees with the findings of Caccia et al. (15). Since there was no evidence of collateral branching (no elevation of FTIR's) it is possible that this increased proportion of type I fibers could be the result of a preferential degeneration of type II fibers. The increased incidence of small diameter fibers in the control group adds to this possibility.

In spite of these observations, this is an unlikely explanation. The gastrocnemius demonstrated a greater corresponding increase in small diameter fibers than the soleus, but with no change in fiber percentage. Furthermore, the observed consistency between muscle fiber pattern and small diameter fiber pattern suggests randomization of fiber atrophy rather than a preferential degeneration of

type II fibers.

It seems reasonable to assume that this trend towards an increased percentage of type I fibers represents a conversion of fiber types, probably brought about by changes in work pattern and load during aging (15, 17, 60). This notion is enhanced by the results of the exercise soleus group, which displayed a significantly larger percentage of type I fibers than either the baseline or control groups. This might be viewed as a more extensive functional modification to a workload requiring primarily slow motor units.

Kugelberg (60) has suggested that primarily phasic muscles, such as the gastrocnemius in this study, may not display the same degree of adaptation as the postural tonic muscles. This may explain the lack of changes observed in the gastrocnemius groups of the present study.

Growth Configurations, ATIR's and FTIR's

In all groups for both muscles, many complex endplates were observed. This complexity was generally characterized by elongation of endplates on muscle fibers and branching of the nerve terminals within the endplate areas. This branching within the endplate was probably the result of growth configurations as described by Tuffery (99). The branching often resembled the morphology of contributions. Pestronk et al. (75) refer to this same process as nerve

terminal sprouting.

Although all muscles in this study displayed these complex endplates, there was an increased incidence with age. The soleus control group demonstrated a greater incidence than the baseline group. The calculated LSR for a difference between the gastrocnemius control group and baseline group was very close to the critical value for significance (0.05 level), suggesting a similar trend in the gastrocnemius. These observations were consistant with those of Tuffery (99) in aging cats. The soleus exercise group also revealed a significantly greater percentage of complex endplates than the baseline or control groups.

The increased nerve terminal sprouting with age supports Tuffery's (99) hypothesis of endplate plate rejuvenation. No real evidence for Barker and Ip's (4) theory of endplate substitution was found. This process would be reflected by an increase in the number of fibers with double endplates which in turn would result in an increased ATIR. This was not seen in the soleus. The gastrocnemius did display a greater ATIR in the exercise group than in either the control or baseline groups. However, this is probably of no practical significance, since the values for all three group ATIR's were well within the normal range for healthy adult humans and rats established by Coors (6).

All FTIR's were also within the normal range. This

indicates no increase in collateral branching beyond what would be expected in healthy adults. This is consistent with the low incidence of small angulated fibers, which are likely denervated and degenerating, and are believed to be the stimulation for collateral sprouting from neighboring fibers (43, 44, 45, 75, 99). More extensive degeneration may be required before an elevation of FTIR's is seen, if at all.

The increased nerve terminal sprouting (growth configurations) in the exercised soleus group raises the question as to whether this phenomenon represents a detrimental or a beneficial effect. The increased terminal sprouting associated with aging alone has been postulated to occur as a replacement process for nerve terminals that are damaged or lost. As a result, the integrity of the neuromuscular junction is maintained (39, 75, 99). The fact that terminal sprouting can be stimulated by treatments that simulate denervation changes in muscle, such as botulinum toxin injection, support this hypothesis (75).

There is also some evidence that this type of change in endplate morphology may be the result of a remodeling process in response to various changes in muscle load, such as changes in body weight, bone length, muscle length, and workload (17, 36, 60). For example, Gerchman observed alterations in staining properties and endplate morphology

in adult rats following 52 days of swimming activity (36).

Cardasis et al. (17) have reported ultrastructural findings that indicate that a continuous remodeling of the neuromuscular junction takes place in the soleus of the adult rat. Additionally, they contend that the process of terminal sprouting and withdrawal may act in concert with one another to sustain or reorganize endplate morphology during adulthood. One process may dominate over the other during periods of growth, aging, and disease. They also suggest that the proportionate increase in type I fibers of the soleus during development, may reflect this endplate remodeling. Kugelberg (60) has also made this suggestion.

The investigators also observed ultrastructural conditions that were unique to the soleus. Predominant among these conditions was the frequent occurrence of unassembled myofibrillar components in the postjunctional sarcoplasm. They hypothesize that these unique conditions may be linked to a preferential endplate plasticity in postural myofibrils. The lack of any significant difference in the gastrocnemius groups of the present study may reflect this preferential plasticity.

Based on the previous discussion, it seems reasonable to assume that the greater incidence of growth configurations observed in the exercise soleus sections may indicate an adaptive response to the increased workload of the muscle. A phenomenon that may be enhanced during aging.

The fact that terminal sprouting maintains the integrity of the neuromuscular junctions, suggests that this plasticity represents a beneficial effect. As mentioned previously, this plasticity may predominate in tonic, postural slow twitch fibers.

There is some question as to whether age changes in skeletal muscle proceed from the motor end plate to the individual fiber or vice versa (34, 43, 44, 45, 101). The observations from the present study offer some indication as to the sequence of these changes. The rather high incidence of terminal sprouting in all groups of both muscles was seen in the absence of extensive degenerative changes in fiber diameters and muscle weight. This finding suggests that the adaptive response of the endplate during aging precedes any major degeneration in muscle fiber morphology.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The present study was initiated in order to observe potential effects of chronic exercise on the morphology of aging skeletal muscle. Relatively few studies have been undertaken to examine these circumstances and there is some question as to the adaptability of skeletal muscle in senescence. In order to accurately characterize the direction and magnitude of any adaptive or degenerative changes, it was decided to examine the subterminal axons and motor endplates on the one hand and the muscle fibers themselves on the other hand.

Seventy five, female Fischer 344, twenty one month old rats were divided into three groups. One group was designated a baseline group and sacrificed immediately. Their tissue served as a standard for comparing age changes following the experimental period. The experimental and control groups were both sacrificed at the end of the exercise training period. The experimental animals progressively trained on a treadmill until they reached an intensity of 23 m/min at an eight degree incline. They ran for thiry minutes a day, five days a week and the entire training program covered twenty weeks. At the end of the twentieth week, the rats were anesthetized and their hearts

and the gastrocnemius and soleus muscle from both limbs were excised. The soleus and gastrocnemius were prepared for light microscopy. Terminal innervation ratios and nerve terminal sprouting were examined to characterize changes in endplate morphology. Histochemical staining for acetyl cholinesterase activity demonstrated neuromuscular junction structure. Differences in fiber diameter and fiber type pattern were studied to gauge morphological changes in the individual fibers. Differences in fiber type were elucidated by staining for myofibrillar ATPase activity.

There was no difference in the absolute heart rates among groups. However, a significantly greater heart weight/body weight ratio ($p<0.01$) (cardiac index) was found. This indicated a relative cardiac hypertrophy and evidence of a resulting exercise training effect.

Mean gastrocnemius weight was not significantly different among groups while the mean exercise soleus weight was significantly less than either the baseline or control groups ($p<0.01$). Similar results were found for the fiber diameter measurements. No difference in mean type I or type II diameters was found among groups for the gastrocnemius. No difference in type I fiber diameters was found among the soleus groups. However, the mean exercise soleus type II diameter was significantly less ($p<0.01$) than the other two groups. This also resulted in a significantly greater type I/type II fiber diameter ratio ($p<0.01$).

The mean small diameter percentages were significantly larger ($p<0.01$) for the exercise and control groups of both muscles than for their corresponding baseline values. Also, the gastrocnemius control group demonstrated a higher incidence of small diameter fiber than the exercise group ($p<0.05$).

The gastrocnemius displayed no difference in fiber type percentages among groups. The soleus exhibited an exercise effect. The exercise group, demonstrated a significantly higher percentage of type I fibers than the other two groups ($p<0.01$).

No practical difference in terminal innervation ratios was established. No age related or exercise induced increase in growth configurations (nerve terminal sprouting) was found for the gastrocnemius. A significant age related increase in terminal sprouting ($p<0.05$) was seen from the soleus baseline to the control group. An exercise effect was also observed resulting in significantly greater nerve terminal sprouting ($p<0.01$) in the exercise group than either of the other two groups.

Conclusions

Based on the foregoing results and discussion, the following conclusions seem tenable:

1) An increased cardiac index in the exercised

animals was interpreted as evidence of a training effect.

2) The soleus demonstrated greater adaptive changes to aging and training than the gastrocnemius. These changes included an apparent conversion of type II fibers to type I fibers (Hypothesis I - a higher percentage of type I fibers following training) and an increased incidence of endplate remodeling characterized by nerve terminal sprouting (Hypothesis III). These changes were probably a result of changes in workload pattern. The greater response of the soleus may be linked to the unique properties of tonic postural muscle fibers and the possibility that it was recruited to a greater degree than the gastrocnemius during the training program.

3) The relatively high incidence in nerve terminal sprouting, in all groups, took place in the absence of any extensive fiber atrophy. This was interpreted as an age induced transformation in endplate morphology that had preceded degenerative changes in the individual muscle fibers.

4) The percentage of small diameter fibers in the exercised gastrocnemius group was less than the respective control group (Hypothesis III). This was interpreted as a reduced rate of atrophy in

the exercised group. The lack of difference in the soleus was attributed to the small incidence of small diameter fibers in all three groups.

5) No age or exercise induced collateral regeneration occurred.

6) The exercise program did not have a negative effect on the senile animals utilized in this study. This was demonstrated by the small differences or lack of differences in heart weights, muscle weights, and fiber diameters among groups.

In order to more completely describe the potential effects of exercise on senile muscular atrophy, further studies are necessary. Based on the results of the present study, it seems that these studies should utilize contrasting training programs, i.e. aerobic vs. anaerobic, over periods of time where a greater degree of age related morphological change can be demonstrated.

BIBLIOGRAPHY

1. Adams, R.D., D. Denny-Brown and C. Perarson. Diseases of the Muscle. A Study in Pathology. 2nd Edition. Hoeber, New York (1962).

2. Anderson, K.L. "Fundamentals of exercise physiology," in 8th Int. Congr. Gerontol. Washington, 1:43-46 (1969).

3. Aniansson, A., G. Grimby, M. Hedberg, A. Rundgren and L. Sperling. "Muscle function in old age." Scand J. Rehab. Med. (suppl.) 6:43-49 (1978).

4. Barker, D. and M. Ip. "The probable existence of a process of motor end plate replacement." J. Appl. Physiol. 176:11-12 (1965).

5. Barnard, R., V. Edgerton and J. Peter. "Effect of exercise on skeletal muscle I: biochemical and histochemical properties." J. Appl. Physiol. 28:762-770 (1970).

6. Bass, A., E. Gutmann and V. Hanzlikova. "Biochemical and histochemical changes in energy supply-enzyme pattern of muscle." Gerontologia 21:31-45 (1975).

7. Bedford, T.G., C.M. Tipton, W.C. Wilson, R.A. Oppliger and C. V. Gisolfi. "Maximum oxygen consumption of rats and its changes with various experimental procedures." J. Appl. Physiol. 47:1278-1283 (1979).

8. Beermann, D.H. "The neural control of skeletal muscle fiber type." Doctoral thesis, Department of Meat and Animal Science, University of Wisconsin-Madison (1976).

9. Beermann, D.H. and R.G. Cassens. "A combined silver and acetylcholinesterase method for staining intramuscular innervation." Stain Tech. 51:173-177 (1976).

10. Beermann, D.H., R.G. Cassens, C.C. Couch and F.J. Nagle. "The effects of experimental denervation and reinnervation of skeletal muscle fiber type and intramuscular innervation." J. Neurol. Sci. 31:207-221 (1977).

11. Birren, J.E. and P.D. Wall. "Age changes in conduction velocity, refractory period, number of fibers, connective tissue space and blood vessels in sciatic nerve of rats." J. Comp. Neur. 104:1-16 (1956).

12. Brooke, M. and K. Kaiser. "Three myosin adenosine triphosphatase systems: the nature of their pH lability and sulfhydral dependence." J. Histochem. and Cytochem. 18:670-672 (1970).

13. Brooks, G. and T. White. "Determination of metabolic and heart rate responses of rats to treadmill exercise." J. Appl. Physiol. 45:1009-1015 (1978).

14. Brown, W.F. "Functional compensation of human motor units in health and disease." J. Neurol. Sci. 20:100-209 (1973).

15. Caccia, M.R., J. Harris and M. Johnson. "Morphology and physiology of skeletal muscle in aging rodents." Muscle and Nerve 2:202-212 (1979).

16. Campbell, M.S., A.J. McComas and F. Petits. "Physiological changes in aging muscles." J. Neurol. Neurosurg. Psychiat. 36:174-182 (1973).

17. Cardasis, A. and H. Padykala. "Ultrastructural evidence indicating reorganization at the neuromuscular junction in the normal rat soleus." Anat. Rec. 200:41-59 (1981)

18. Carlson, K.E., W. Alston and D.S. Feldman. "Electromyographic study of aging in skeletal muscle." Am. J. Phys. Med. 43:141-145 (1964).

19. Close, R.I. "Dynamic properties of mammalian skeletal muscles." Physiol. Rev. 52:129-197 (1972).

20. Coers, C. and A.L. Wolf. The Innervation of Muscle. A Biopsy Study. Oxford: Blackwell Scientific Publications (1959).

21. Coers, C., N. Telermann-Toppet and J. Gerard. "Terminal innervation ratios in neuromuscular disease. I. Methods and controls." Arch. Neurol. 29:210-214 (1973).

22. Coers, C., N. Telermann-Toppet and J. Gerard. "Terminal innervation ratios in neuromuscular disease. II. Disorders of lower motor neuron,

peripheral nerve, and muscle." Arch. Neurol. 29:215-222 (1973).

23. Crews, E., L. Fuge, L. Oscai, J. Holloszy and R. Shank. "Weight, food intake, and body composition: effects of exercise and of protein deficiency." Am. J. Physiol. 216:359-363 (1969).

24. deVries, H.A. "Efficiency of electrical activity as a measure of the functional state of muscle tissue." Am. J. Phys. Med. 47:10-22 (1968).

25. deVries, H.A. "Physiological effects of an exercise training regimen upon men aged 52-88." J. Geront. 25:325-336 (1970).

26. deVries, H.A. "Physiology of exercise and aging." In: Scientific Perspectives and Social Issues, D.S. Woodruff and J.E. Birren (eds.). New York: D. Van Nostrand Co. (1978).

27. Denny-Brown, D. "Experimental studies pertaining to hypertrophy, regeneration and degeneration." Res. Publ. Assoc. Nerve and Ment. Dis. 38:147-196 (1960).

28. Drahota, Z. and E. Gutmann. "The effect of age on compensatory and post-functional hypertrophy in cross-striated muscle." Gerontologia 6:81-90 (1962).

29. Edgerton, U., L. Gerchman and R. Carrow. "Histochemical changes in rat skeletal muscle after exercise." Exp. Neurol. 24:110-123 (1969).

30. Edds, M. Collateral nerve regeneration." Quart. Rev. Biol. 28:260-276 (1953).

31. Engle, W.K. "Fiber type nomenclature of human skeletal muscle for histochemical purposes." Neurol. (Minneap.) 24:344-348 (1974).

32. Ferguson, G. Statistical Analysis in Psychology and Education. New York: McGraw-Hill Co. (1971).

33. Fitts, R.H. "Aging in skeletal muscle." In: Aging and Exercise: A Scientific Approach, E. Smith and R. Serfass (eds.). Enslow Publishers: Hillside, NJ (1981).

34. Frolkis, V.V., O.A. Martynenko and U.P. Zamostyan.

"Aging of the muscular apparatus." Gerontology 22:244-279 (1976).

35. Fujisawa, K. "Some observations on the skeletal musculature of aged rats: Part 1 - Histological aspects." J. Neurol. Sci. 22:353-366 (1974).

36. Gerchman, L. "Effects of long term physical exercise upon the morphology and histochemistry of the ventral motor neuron." Anat. Rec. 160:352 (1968).

37. Goodrick, C.L. "Effects of long-term voluntary wheel exercise on male and female wistar rats." Gerontol. 26:22-33 (1980).

38. Gutmann, E. and V. Hanzlikova. "Age changes of motor end plates in muscle fibers of the rat." Gerontologia 11:12-24 (1965).

39. Gutmann, E. and V. Hanzlikova. "Motor unit in old age." Nature 209:921-922 (1966).

40. Gutmann, E., V. Hanzlikova and B. Sakoubek. "Changes in the neuromuscular system during old age." Exp. Geront. 3:141-146 (1968).

41. Gutmann, E. "Nervous and hormonal mechanisms in the aging process." Exp. Geront. 5:357-366 (1970).

42. Gutmann, E., V. Hanzlikova and F. Vyskocil. "Age changes in cross striated muscle of the rat." J. Physiol. 219:331-343 (1971).

43. Gutmann, E. and V. Hanzlikova. Age Changes in the Neuromuscular System. pp. 1-195. Scientechnica: Bristol (1972).

44. Gutmann, E. and V. Hanzlikova. "Basic mechanisms of aging in the neuromuscular system." Mech. Age. Dev. 1:327-349 (1972/1973).

45. Gutmann, E. "Maintenance and formation of neuromuscular synapses in senescent muscle." Activ. Nerv. Sup. 18:137-138 (1976).

46. Gutmann, E. and V. Hanzlikova. "Fast and slow motor units in aging." Gerontology 22:280-300 (1976).

47. Gutmann, E. "Muscle." In: Handbook of the Biology of Aging, C.C. Finch and L. Hayflick (eds.). New York: Van Nostrand Reinhold Co. (1977).

48. Hanzlikova, V., S. Schiaffino and P. Settembrini. "Histochemical fiber type characteristics in the normal and the persistent levator ani muscle of the rat." Histochemie 22:45-50 (1970).

49. Hariiman, D., D. Taverner and A. Woolf. "Ekbom's syndrome and burning paresthesiae: A biopsy study by vital staining and electron microscopy of the intramuscular innervation with a note on age changes in motor nerve endings in distal muscles." Brain 93:393-406 (1970).

50. Hoffman, H. "Survival distributions for selected laboratory rat strains and stocks." In: Development of the Rodent as a Model System of Aging, D. Gibson, R. Adelman and C. Finch (eds.). U.S. Dept. of Health, Education and Welfare.

51. Hollman, W. "Changes in the capacity for maximal and continuous effort in relation to age." In: International Sports and Physical Education, E. Jokl and E. Simon (eds.). Springfield: C.C. Thomas (1964).

52. Ianuzzo, C., P. Patel, U. Chen and P. O'Brien. "A possible trophic influence on fast and slow skeletal muscle myosin." In: Plasticity of Muscle, D. Pette (ed.). Berlin - New York: Walter de Gruyter Co. (1980).

53. Janson, J., W. Thompson and D. Kuffler. "The formation of synaptic connections as illustrated by studies of the neuromuscular junction." In: Maturation of the Nervous System. Prog. Brain Res., M. Conner, R. Baker, W. Van De Poll, D. Swaab and H. Uylings (eds.). Amsterdam: Elsevier Scientific Publishing Co. 48:3-18.

54. Jennekens, F.G., B.E. Tomlinson and J.W. Walton. "Histochemical aspects of five limb muscles in old age. An autopsy study." J. Neurol. Sci. 14:259-276 (1971).

55. Karvonen, M.J. "The aging endurance athlete." In: 8th Int. Congr. Gerontol., Washington, 1:40-42 (1969).

56. Kelley, S.S. "The effect of age on neuromuscular transmission." J. Physiol. 274:51-62 (1978).

57. Kiessling, K.H., L. Pilstrom, A. Bylund, B. Sultin and

K. Diehl. "Enzyme activities and morphometry in skeletal muscle of middle-aged men after training." Scand. J. Clin. Lab. Invest. 33:63-69 (1974).

58. Kiernan, J.A. "An explanation of axonal regeneration in peripheral nerves and its failure in the central nervous system." Med. Hyp. 4:15-26 (1978).

59. Kiernan, J.A. "Hypotheses concerned with axonal regeneration in the mammalian nervous system." Biol. Rev. 54:155-197 (1979).

60. Kugelberg, E. "Adaptive transformation of rat soleus motor units during growth." J. Neurol. Sci. 27:269-289 (1976).

61. Larsson, L. and J. Karlsson. "Isometric and dynamic endurance as a function of age and skeletal muscle characteristics." Acta Physiol. Scand. 104:129-136 (1978).

62. Larsson, L., B. Sjodin and S. Karlsson. "Histochemical and biochemical changes in human skeletal muscle with age in sedentary males, age 22-65 years." Acta Physiol. Scand. 103:31-39 (1978).

63. Larsson, L., G. Grimby and J. Karlsson. "Muscle strength and speed of movement in relation to age and muscle morphology." J. Appl. Physiol. 46:452-456 (1979).

64. Magladery, J.W. "Neurophysiology of aging." In: Handbook of Aging and the Individual, J.E. Birren (ed.). University of Chicago Press, pp. 173-186 (1959).

65. McCafferty, W.B. and D.W. Edington. "Skeletal muscle and organ weights of aged and trained male rats." Gerontology 20:44-50 (1970).

66. McCarter, R. "Effects of age on contraction of mammalian skeletal muscle." In: Aging (Vol. 6): Aging in muscle, G. Kaldor and W. DiBattista (eds.). Raven Press: New York (1978).

67. McComas, A.J., A.R.M. Upton and R.E.P. Sica. "Motor neurone disease and ageing." Lancet 1477-1480 (1973).

68. Morehouse, L.E. "Physiological basis of strength development." In: Exercise and Fitness, Library of Congress 60-11563, 193-199 (1959).

69. Moritani, T. "Electromyographic analysis of muscle strength gains: neural and hypertrophic effects." Nat. Strength Coac. Assoc. J. 1:32-37 (1978).

70. Moritani, T. and H.A. deVries. "Neural factors versus hypertrophy in the time course of muscle strength gain." Am. J. Phys. Med. 58:115-130 (1979).

71. Moritani, T. and H.A. deVries. "Potential for gross muscle hypertrophy in older men." J. Geront. 35:672-682 (1980).

72. Moritani, T. "Training adaptions in the muscles of older men." In: Aging and Exercise: A Scientific Approach, E. Smith and R. Serfass (eds.). Hillside, NJ: Enslow Publishers (1981).

73. Orlander, J., K. Kiessling, L. Larsson, J. Karlsson and A. Anianson. "Skeletal muscle metabolism and ultrastructure in relation to age in sedentary men." Acta Physiol. Scand. 104:249-261 (1978).

74. Padykula, H. and E. Herman. "The specificity of the histochemical method for adenosin triphosphatase." J. Histochem. and Cytochem. 3:170-195 (1955).

75. Pestronk, A., D. Drackman and J. Griffin. "Effects of aging on nerve sprouting and regeneration." Exp. Neurol. 70:65-82 (1980).

76. Peterson, R. "Microstructural and ultrastructural response of myocardium to aerobic physical training." Unpublished Doctoral Thesis, University of Wisconsin-Madison (1971).

77. Retzlaff, E. and J. Fontaine. "Functional and structural changes in motor neurons with age." In: Behavior, Aging and the Nervous System, A.T. Welford and J.E. Birren (eds.). Springfield: C.C. Thomas, pp. 340-352 (1965).

78. Roberts, J., S. Baskin and P. Goldberg. "Age changes in the neuromuscular system of rats." Exp. Aging Res. 3:75-84 (1977).

79. Rothstein, M. "Aging and the alteration of enzymes: a review." Mech. Age Dev. 4:325-338 (1975).

80. Rowe, R.W.D. "The effect of senility on skeletal muscles in the mouse." Exp. Geront. 4:119-126 (1969).

81. Rubinstein, J.L. "Ageing changes in muscle." In: Structure and Function of Muscle, London - New York: Academic Press, 3:209-226 (1960).

82. Saltin, B., J. Henriksson, E. NyGaard, P. Andersen and E. Jansson. "Fiber types and metabolic potentials of skeletal muscles in sedentary man and endurance runners." In: The Marathon: Physiological, Medical, Epidemiological, and Psychological Studies, P. Miluy (ed.). Annals of the New York Academy of Sciences 301:1977.

83. Scelsi, R., C. Marchetti and P. Poggi. "Histochemical and ultrastructural aspects of m. vastus laterelis in sedentary old people (age 65-89 years)." Acta Neuropathol. (Berlin) 51:99-105 (1980).

84. Shepherd, R. and P. Gollnick. "Oxygen uptake of rats at different work intensities." Pflugers Arch. 362:219-222 (1976).

85. Smith, D. "Reduced capabilities of synaptic transmission in aged rats." Exp. Neurol. 66-650-666 (1979).

86. Sokal, R. and F. Rohlf. Biometry, San Francisco: W.H. Freeman and Co. (1969).

87. Soukup, T., J. Uydra and M. Cerny. "Changes in ATPase and SDH reactions of the rat extrafusal and intrafusal muscle fibers after preincubations at different pH." Histochemistry 60:71-84 (1979).

88. Stevenson, J., B. Box, U. Feleki and J. Beaton. "Bouts of exercise and food intake in the rat." J. Appl. Physiol. 21:118-122 (1966).

89. Suominen, H., E. Heikkinen and T. Parkatti. "Effect of eight weeks of physical training on muscle and connective tissue on the m. vastus lateralis in 69 year old men and women." J. Geront. 32:33-37 (1977).

90. Suzuki, A. and R. Cassens. "pH sensitivity of myosin adenosine triphosphatase and subtypes of myofibers in porcine muscle." Histochem. J. 12:687-693

(1980).

91. Syrovy, I. and E. Gutmann. "Changes in speed of contraction and ATPase activity in striated muscle during old age." Exp. Geront. 5:31-35 (1970).

92. Tauchi, H., T. Yoshioka and H. Kobayashi. "Age change of skeletal muscles of rats." Gerontologia 17:219-227 (1971).

93. Tesch, P. "Muscle fatigue in man." Acta Physiol. Scand. Suppl. 480 (1980).

94. Tohgi, H., T. Shimizu, K. Inoue and M. Kameyama. "Quantitative histochemical studies of age changes of human muscles I: Histograms of fiber diameters." Clin. Neurol. (Tokyo) 15:791-797 (1975).

95. Toghi, H., T. Shimizu, K. Inoue and M. Kameyama. "Quantitative histochemical studies on age changes of human muscles II: The proportion of fiber types and type grouping." Clin. Neurol. (Tokyo) 15:798-804 (1975).

96. Tomanek, R.J. and Y.K. Wood. "Compensatory hypertrophy of the plantaris muscle in relation to age." J. Geront. 25:23-29 (1970).

97. Tomlinson, B.E., J.N. Walton and J.J. Rebeiz. "The effects of aging and of cachexia upon skeletal muscle: A histopathological study." J. Neur. Sci. 9:321-346 (1969).

98. Tomonaga, M. "Histochemical and ultrastructural changes in senile human skeletal muscle." J. Am. Ger. Soc. 25:125-131 (1977).

99. Tuffery, A.R. "Growth and degeneration of motor endplates in normal cat hind limb muscles." J. Anat. 110:221-247 (1971).

100. Van Liere, E. and D. Northrup. "Cardiac hypertrophy produced by exercise in albino and hooded rats." J. Appl. Physiol. 11:91-92 (1957).

101. Van Steenis, G. and R. Kroes. "Changes in the nervous system and musculature of old rats." Vet. Path. 8:320-332 (1971).

102. Visser, S.L. and W. deRuke. "Influence of sex and age on EMG contraction pattern." Europ. Neurol.

12:229-235 (1974).

103. Yiengst, M., C. Barrows and W. Shock. "Age changes in chemical composition of muscle and liver in the rat." J. Gerontol. 14:400-404 (1959).

APPENDIX

Table I

Combined Silver and Acetylcholinesterase Method for Staining Intramuscular Innervation (9)

Preparation of tissue for staining:

1. At 20° C cut fresh frozen sections 30-100 μ thick.
2. Mount sections on cooled glass slides coated with ethylenediaminetetraacetic acid (EDTA).
3. Allow sections to thaw and dry at room temperature.
4. Fix dried sections in cold 30% formalin for 15 minutes.

Staining of the axons and motor end plates:

1. Dissolve 20 mg acetylthiocholine iodide in a few drops of H_2O. Add 10 ml stock solution.

   Acetylcholinesterase stock solution

Copper sulphate ($CuSO_4 \cdot 5\ H_2O$)	0.3 g
Glycine	0.375 g
Magnesium chloride	1.00 g
Maleic acid	1.75 g
4% NaOH	30 ml
40% Na_2SO_4 (hot saturated)	170 ml

   This pH 6.0 solution keeps indefinitely stored at 37° C.

2. Incubate sections 10-50 min at 37° C (15 min was found to be optimal for most tissues.)
3. Rinse in three changes of saturated Na_2SO_4.
4. Rinse in distilled H_2O.
5. Incubate sections in 0.5 M $K_3Fe(CN)_6$ at room temperature for 10 minutes.
6. Rinse in running tap water for 10 minutes.
7. Rinse in distilled H_2O.
8. Impregnate with 10% $AgNO_3$ at room temperature for 60 minutes.
9. Rinse in distilled water for 5 minutes with agitation.

Table I (cont'd)

10. Reduce in Bodian's developer 0.5 to 2.5 minutes. (Note: This step is critical to the end result and requires close visual observation.)

Bodian's developer

Sodium sulphite (anhydrous)	5.0 g
Hydroquinone	1.0 g
Distilled H_2O	to 100 ml

11. Rinse in acetic acid for 2 minutes.

12. Rinse in distilled H_2O for 5 minutes.

13. Dehydrate, clear and mount.

Table II

Modified Method for Myosin Adenosine Triphosphatase (ATPase) (90)

---

1. Dry cryostat sections (8-10 μ) for 30-40 min at room temperature.

2.1. Alkaline preincubation

Preincubate at room temperature (19-21° C) for 20 min in the following solution:

Alkali preincubating solution

0.1 M Sodium barbital	20 ml
0.18 M $CaCl_2$	25 ml
Distilled water	55 ml

Adjust pH to 10.4 with 0.1 M NaOH just before preincubation.

Change solution three times during preincubation.

2.2. Acid preincubation

Preincubate at room temperature (19-21° C) for 5 min in 0.2 M barbital acetate buffer.

A:	Sodium acetate	1.94 g
	Sodium barbital	2.94 g
	Distilled water	100 ml
B:	0.1 M HCl	
	Solution A	20 ml
	Solution B	45 ml
	Distilled water	32 ml

Adjust pH to 4.3 with solution B.

3. Wash sections preincubated in acid solution in the alkali preincubating solution (9.4-9.5) for about 5 seconds.

4. Incubate the sections preincubated in alkali solution for 30 min and the sections preincubated in acid solution for 45 min at room temperature (19-21° C) in the following solution:

Table II (cont'd)

Incubating solution

ATP	90 mg
0.1 M barbital	12 ml
0.18 M $CaCl_2$	6 ml
Distilled water	40 ml

Adjust pH to 9.4 with 0.1 M NaOH before incubation.

5. Wash in three changes of 1% $CaCl_2$ for 10 min.

6. Transfer to 2% $CoCl_2$ (or $Co(NO_3)_2$) for 3-5 min.

7. Wash in six changes of 0.2% sodium barbital.

8. Wash in four changes of distilled water.

9. Place in 1-5% yellow ammonium sulfide for 15-20 seconds.

10. Wash in four changes of distilled water.

11. Place in the fixative (1 volume acetic acid + 3 volumes absolute ethanol for 2-5 min.

12. Place in absolute ethanol (2 changes) for 2-5 min.

13. Place in two changes of xylene for 5 minutes each.

14. Mount in permount.

TITLE OF THESIS The Effect of Exercise on Morphological Changes Associated With Senile Muscular Atrophy

MAJOR PROFESSOR William G. Reddan, Ph.D.

MAJOR DEPARTMENT Physical Education

MINOR(S) Distributed

NAME Charles Lewis Stebbins

PLACE AND DATE OF BIRTH Denver, Colorado 12/26/49

COLLEGES AND UNIVERSITIES: YEARS ATTENDED AND DEGREES

California State University at Hayward	1967-1972	B.S.
University of California-Berkeley	1973-1974	M.A.
University of Wisconsin-Madison	1975-1981	Ph.D.

MEMBERSHIPS IN LEARNED OR HONORARY SOCIETIES

American College of Sports Medicine

PUBLICATIONS

DATE August 24, 1981